US009189677B2

(12) **United States Patent**
Oda et al.

(10) **Patent No.: US 9,189,677 B2**
(45) **Date of Patent: *Nov. 17, 2015**

(54) **RECORDING MEDIUM HAVING OBSERVATION PROGRAM RECORDED THEREIN AND OBSERVATION APPARATUS**

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(73) Assignee: **PANASONIC HEALTHCARE HOLDINGS CO., LTD.** (JP)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 357 days.

This patent is subject to a terminal disclaimer.

(21) Appl. No.: **13/729,264**

(22) Filed: **Dec. 28, 2012**

(65) **Prior Publication Data**

US 2013/0188033 A1 Jul. 25, 2013

Related U.S. Application Data

(63) Continuation of application No. PCT/JP2011/079763, filed on Dec. 22, 2011.

(30) **Foreign Application Priority Data**

Feb. 28, 2011 (JP) 2011-042418

(51) **Int. Cl.**
H04N 9/47 (2006.01)
G06K 9/00 (2006.01)
(Continued)

(52) **U.S. Cl.**
CPC ***G06K 9/00134*** (2013.01); ***G01N 15/1463*** (2013.01); ***G02B 21/0088*** (2013.01); ***G02B 21/365*** (2013.01); *G01N 2015/1493* (2013.01); *G02B 21/0056* (2013.01)

(58) **Field of Classification Search**
USPC 348/79, 46; 382/128, 133; 250/288, 282
See application file for complete search history.

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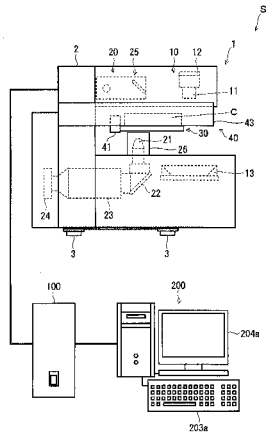
Primary Examiner — Gims Philippe
(74) *Attorney, Agent, or Firm* — Cantor Colburn LLP

(57) **ABSTRACT**

A recording medium having an observation program recorded therein, the program may cause a computer to execute: an entire-image-pickup process of picking up an image of a sample by picking up an image of an entire container containing the sample and a solution; a sample-mass-identification process of identifying a sample mass having the samples gathering therein, from the image picked up in the entire image-pickup process; a sample-mass-determination process of extracting shape information of the identified sample mass, and determining a state of the sample mass based on the shape information; a coordinate-detection process of selecting a magnifying-observation-target sample mass from the identified sample masses, and detecting coordinates of the center of the magnifying-observation-target sample mass.

14 Claims, 17 Drawing Sheets



(51) **Int. Cl.**

G01N 15/14	(2006.01)
G02B 21/00	(2006.01)
G02B 21/36	(2006.01)

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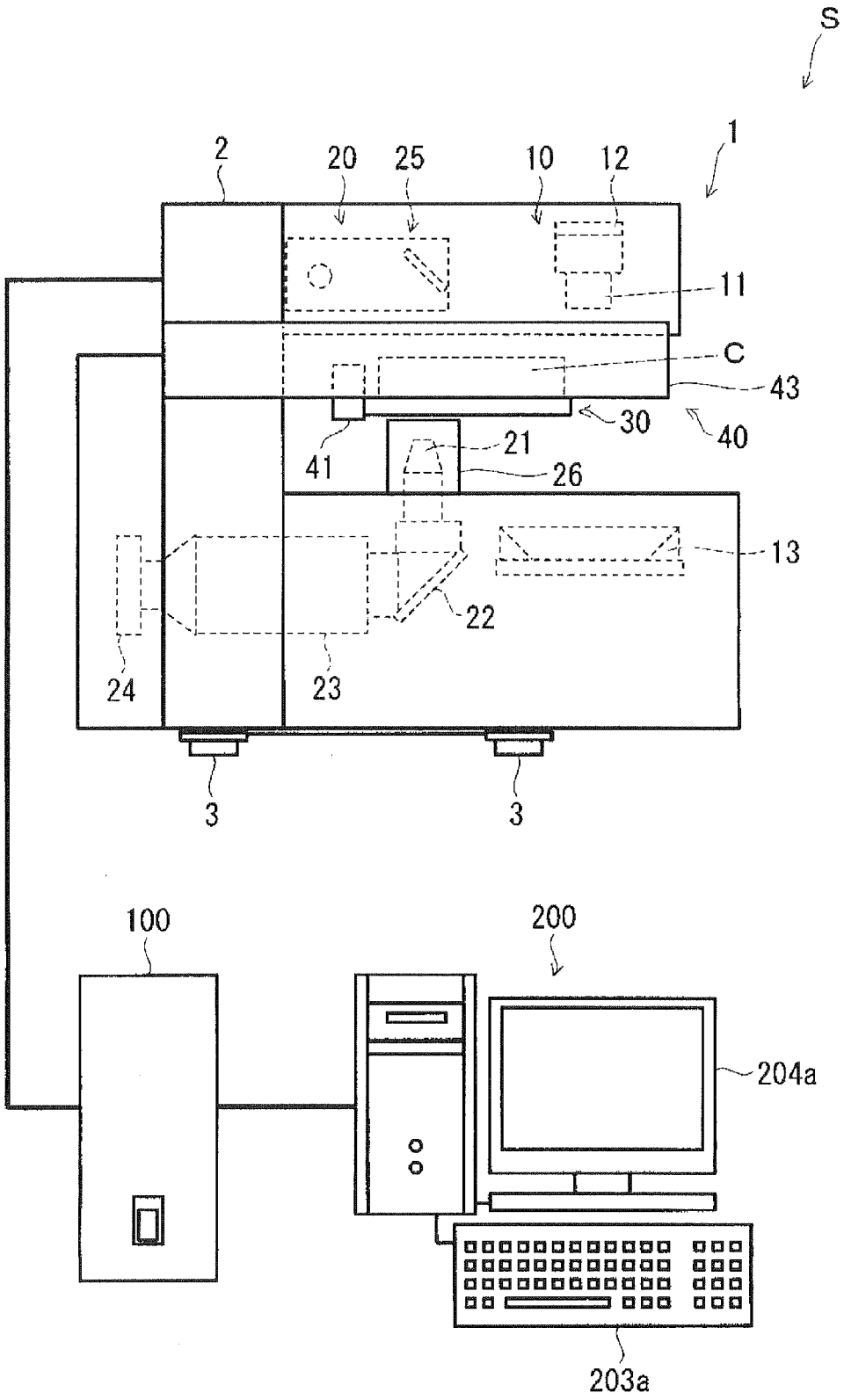
S
1
2
20
25
10
12
11
C
43
30
40
41
21
26
13
22
24
23
3
3
100
200
204a
203a

FIG. 1

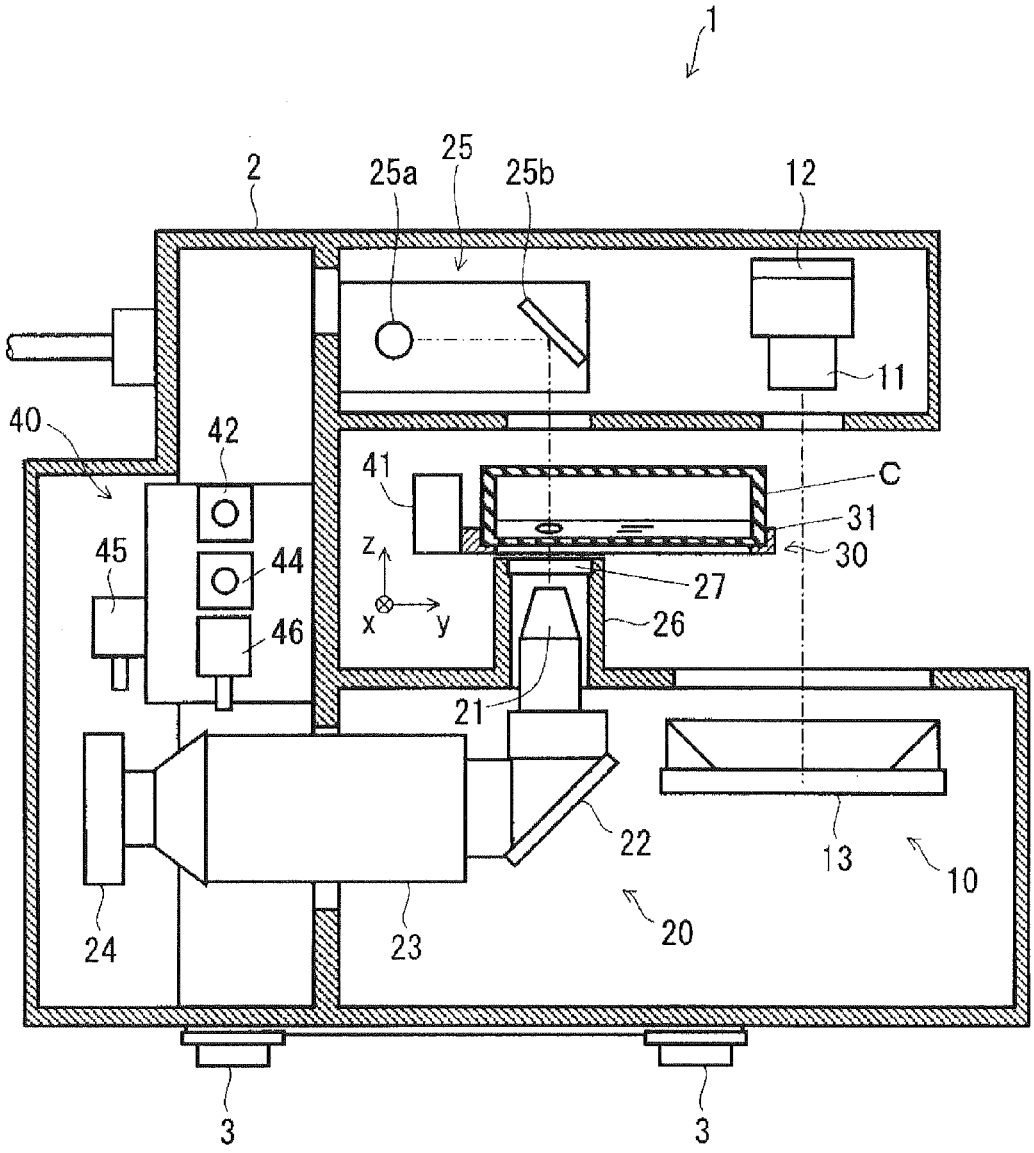
1
2
25a
25
25b
12
11
40
42
41
C
31
30
45
44
z
27
x
y
46
26
21
22
13
10
20
24
23
3
3

FIG. 2

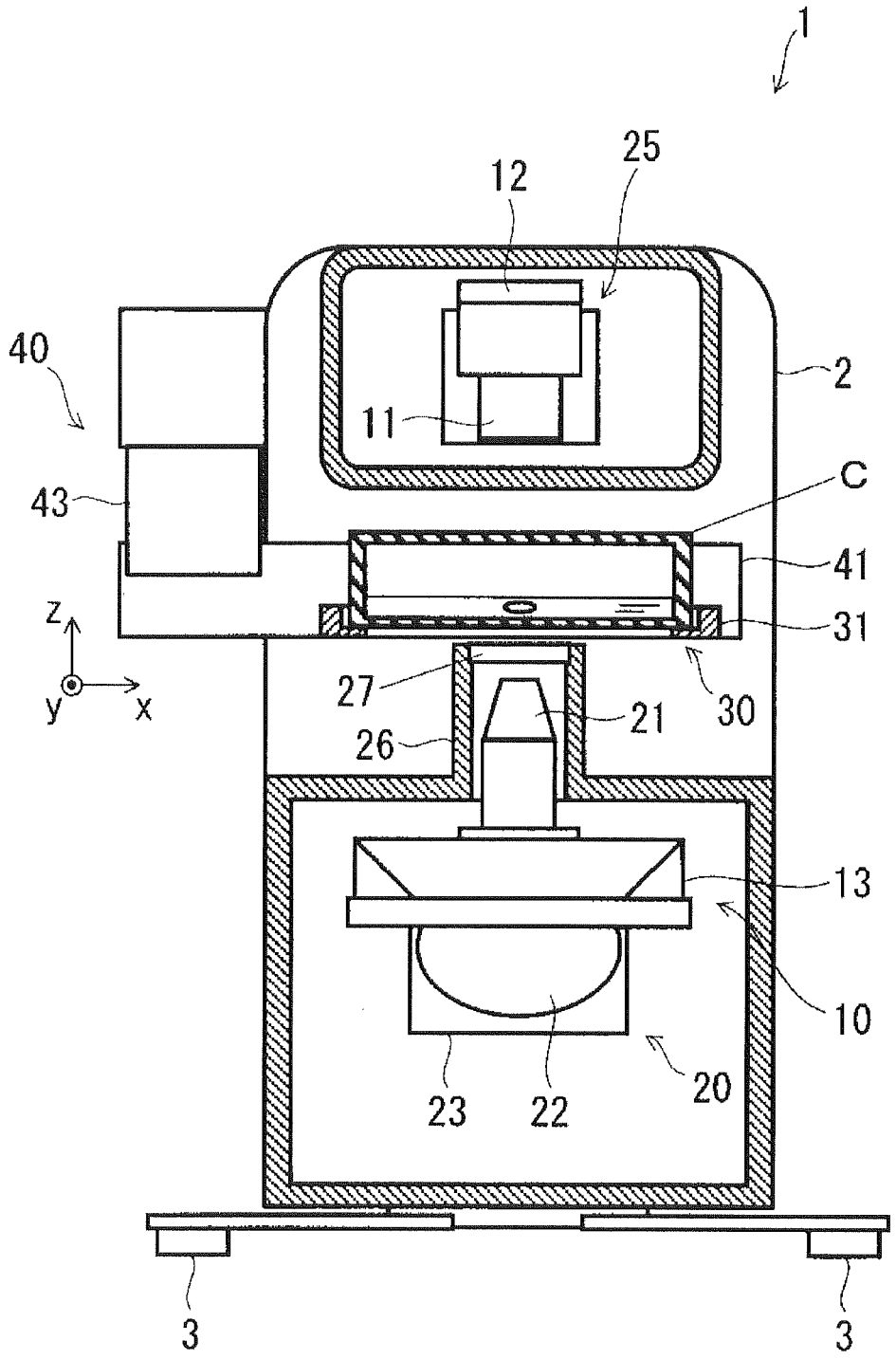

FIG. 3

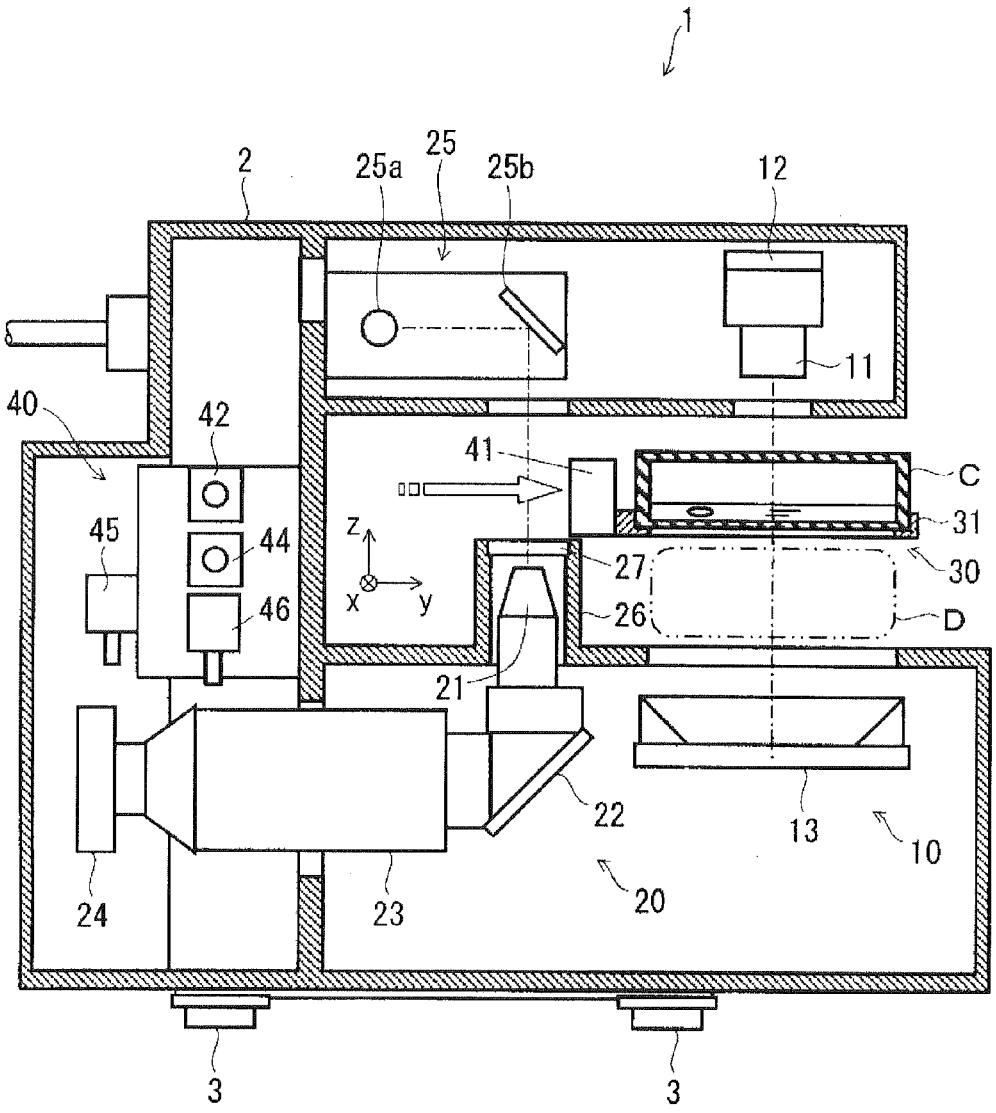
1
2
25a
25
25b
12
11
40
42
41
C
31
45
44
z
27
30
46
x
y
26
D
21
22
13
10
24
23
20
3
3

FIG. 4

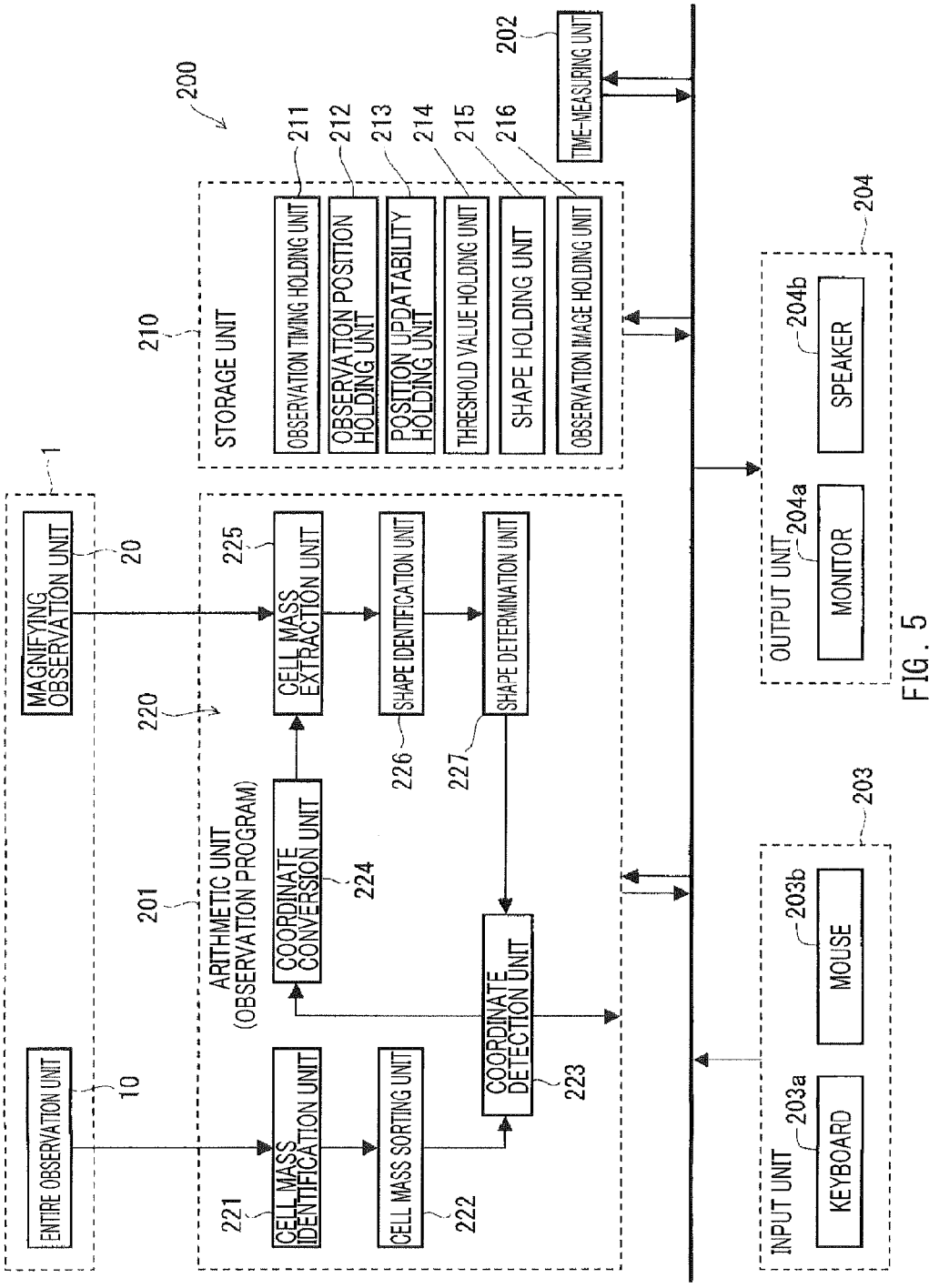
1
ENTIRE OBSERVATION UNIT
10
MAGNIFYING OBSERVATION UNIT
20
200
ARITHMETIC UNIT (OBSERVATION PROGRAM)
201
220
CELL MASS IDENTIFICATION UNIT
221
CELL MASS SORTING UNIT
222
COORDINATE DETECTION UNIT
223
COORDINATE CONVERSION UNIT
224
CELL MASS EXTRACTION UNIT
225
SHAPE IDENTIFICATION UNIT
226
SHAPE DETERMINATION UNIT
227
210
STORAGE UNIT
OBSERVATION TIMING HOLDING UNIT
211
OBSERVATION POSITION HOLDING UNIT
212
POSITION UPDATABILITY HOLDING UNIT
213
THRESHOLD VALUE HOLDING UNIT
214
SHAPE HOLDING UNIT
215
OBSERVATION IMAGE HOLDING UNIT
216
TIME-MEASURING UNIT
202
INPUT UNIT
203
KEYBOARD
203a
MOUSE
203b
OUTPUT UNIT
204
MONITOR
204a
SPEAKER
204b

FIG. 5

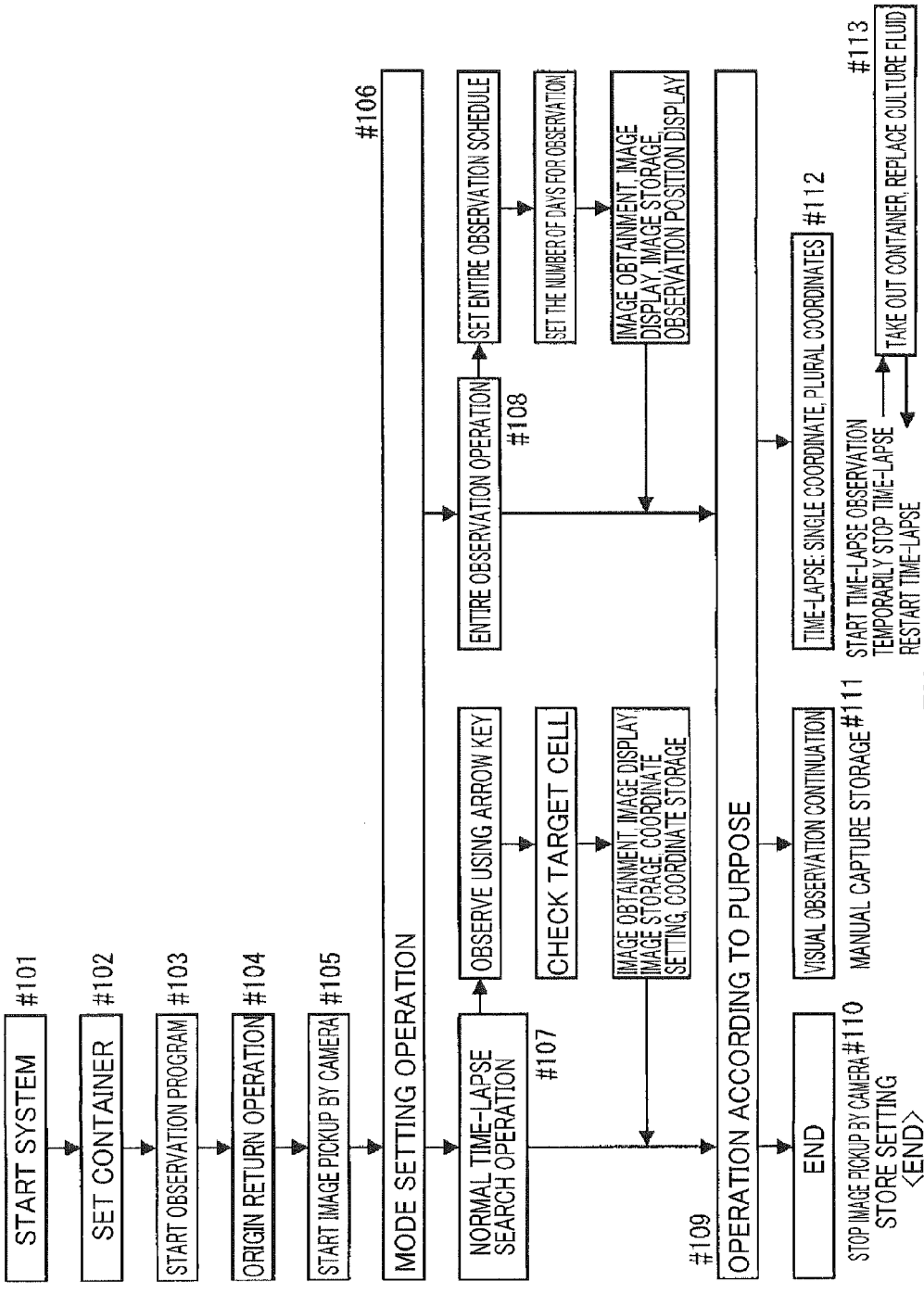
START SYSTEM
#101
SET CONTAINER
#102
START OBSERVATION PROGRAM
#103
ORIGIN RETURN OPERATION
#104
START IMAGE PICKUP BY CAMERA
#105
MODE SETTING OPERATION
#106
NORMAL TIME-LAPSE SEARCH OPERATION
#107
OBSERVE USING ARROW KEY
CHECK TARGET CELL
IMAGE OBTAINMENT, IMAGE DISPLAY, IMAGE STORAGE, COORDINATE SETTING, COORDINATE STORAGE
ENTIRE OBSERVATION OPERATION
#108
SET ENTIRE OBSERVATION SCHEDULE
SET THE NUMBER OF DAYS FOR OBSERVATION
IMAGE OBTAINMENT, IMAGE DISPLAY, IMAGE STORAGE, OBSERVATION POSITION DISPLAY
#109
OPERATION ACCORDING TO PURPOSE
END
STOP IMAGE PICKUP BY CAMERA #110
STORE SETTING
<END>
VISUAL OBSERVATION CONTINUATION
MANUAL CAPTURE STORAGE #111
TIME-LAPSE: SINGLE COORDINATE, PLURAL COORDINATES
#112
START TIME-LAPSE OBSERVATION
TEMPORARILY STOP TIME-LAPSE
RESTART TIME-LAPSE
#113
TAKE OUT CONTAINER, REPLACE CULTURE FLUID

FIG. 6

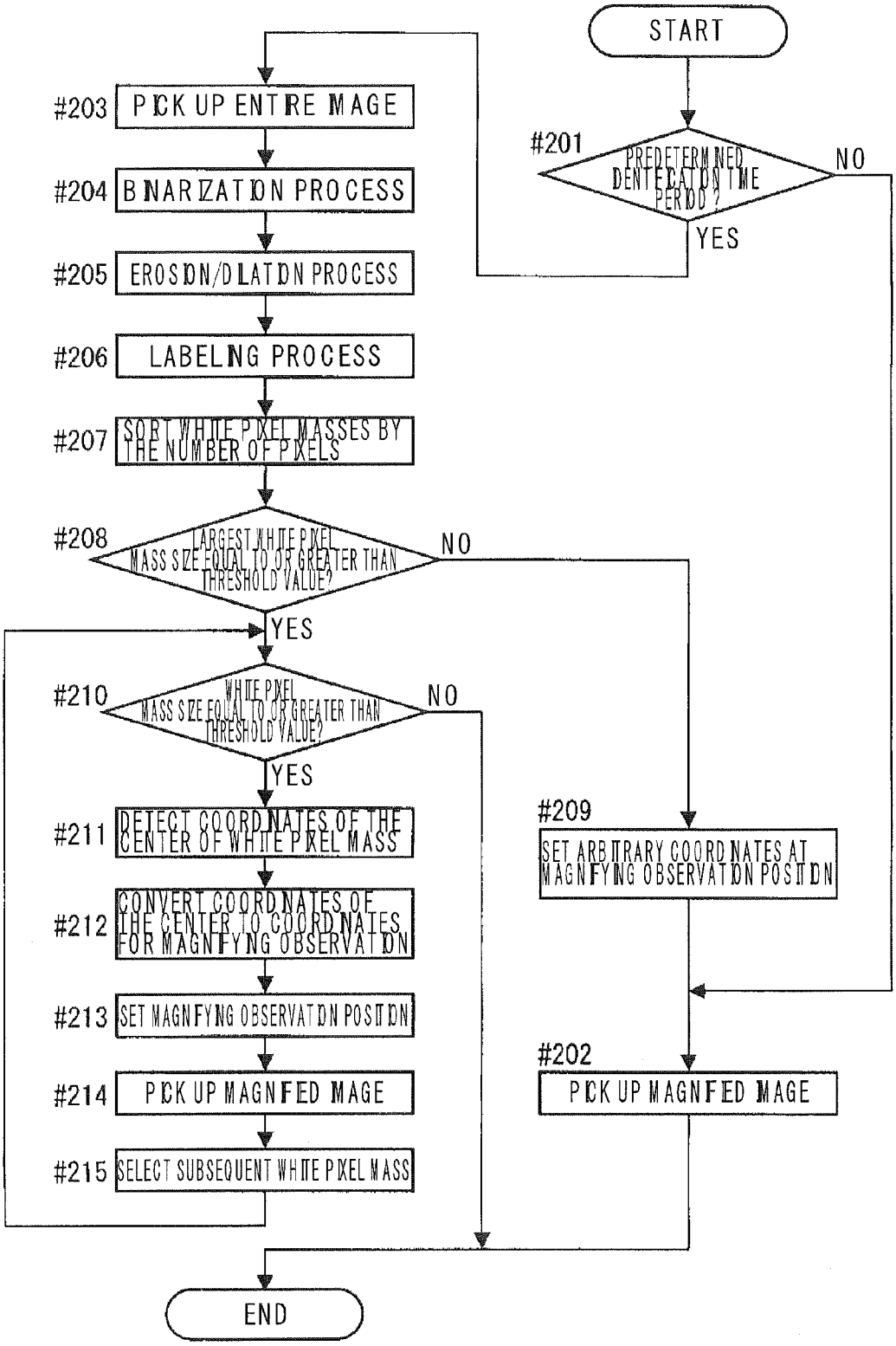
START
#201
PREDETERMINED DETECTION TIME PERIOD ?
NO
YES
#203
PICK UP ENTIRE IMAGE
#204
BINARIZATION PROCESS
#205
EROSION/DILATION PROCESS
#206
LABELING PROCESS
#207
SORT WHITE PIXEL MASSES BY THE NUMBER OF PIXELS
#208
LARGEST WHITE PIXEL MASS SIZE EQUAL TO OR GREATER THAN THRESHOLD VALUE?
NO
YES
#210
WHITE PIXEL MASS SIZE EQUAL TO OR GREATER THAN THRESHOLD VALUE?
NO
YES
#211
DETECT COORDINATES OF THE CENTER OF WHITE PIXEL MASS
#212
CONVERT COORDINATES OF THE CENTER TO COORDINATES FOR MAGNIFYING OBSERVATION
#213
SET MAGNIFYING OBSERVATION POSITION
#214
PICK UP MAGNIFIED IMAGE
#215
SELECT SUBSEQUENT WHITE PIXEL MASS
#209
SET ARBITRARY COORDINATES AT MAGNIFYING OBSERVATION POSITION
#202
PICK UP MAGNIFIED IMAGE
END

FIG. 7

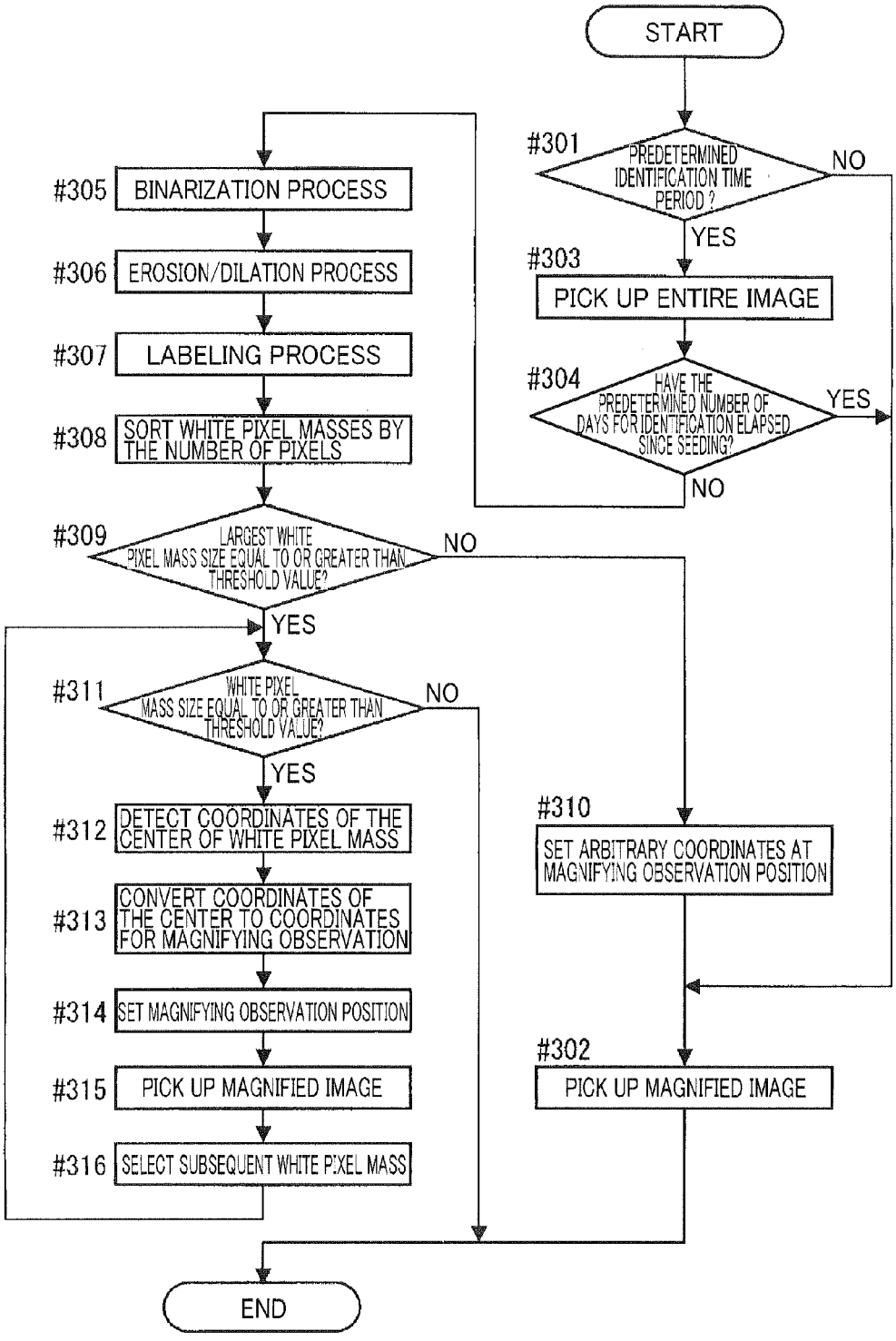
START
#301
PREDETERMINED IDENTIFICATION TIME PERIOD ?
NO
YES
#303
PICK UP ENTIRE IMAGE
#304
HAVE THE PREDETERMINED NUMBER OF DAYS FOR IDENTIFICATION ELAPSED SINCE SEEDING?
YES
NO
#305
BINARIZATION PROCESS
#306
EROSION/DILATION PROCESS
#307
LABELING PROCESS
#308
SORT WHITE PIXEL MASSES BY THE NUMBER OF PIXELS
#309
LARGEST WHITE PIXEL MASS SIZE EQUAL TO OR GREATER THAN THRESHOLD VALUE?
NO
YES
#311
WHITE PIXEL MASS SIZE EQUAL TO OR GREATER THAN THRESHOLD VALUE?
NO
YES
#312
DETECT COORDINATES OF THE CENTER OF WHITE PIXEL MASS
#313
CONVERT COORDINATES OF THE CENTER TO COORDINATES FOR MAGNIFYING OBSERVATION
#314
SET MAGNIFYING OBSERVATION POSITION
#315
PICK UP MAGNIFIED IMAGE
#316
SELECT SUBSEQUENT WHITE PIXEL MASS
#310
SET ARBITRARY COORDINATES AT MAGNIFYING OBSERVATION POSITION
#302
PICK UP MAGNIFIED IMAGE
END

FIG. 8

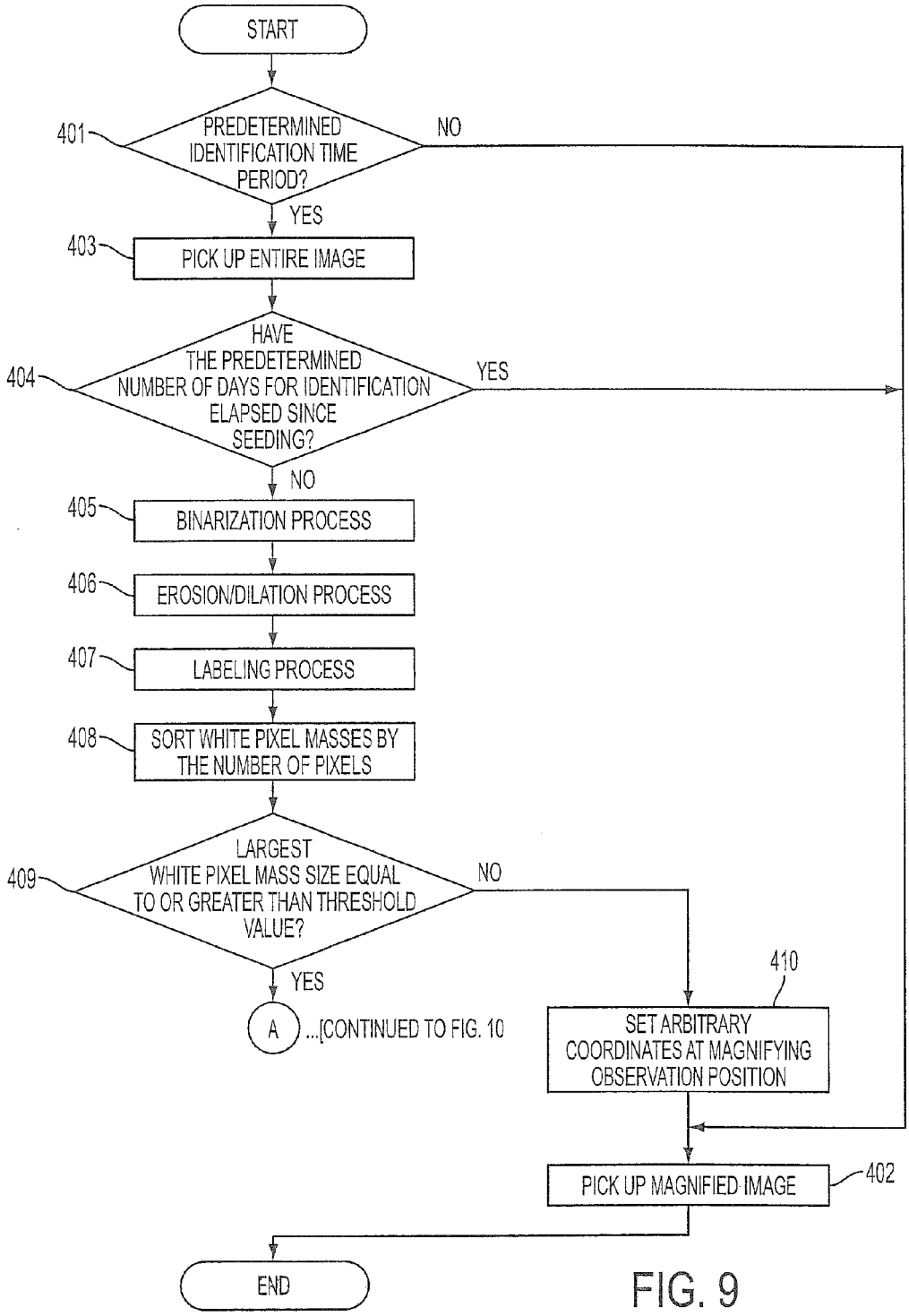
START
401
PREDETERMINED IDENTIFICATION TIME PERIOD?
NO
YES
403
PICK UP ENTIRE IMAGE
404
HAVE THE PREDETERMINED NUMBER OF DAYS FOR IDENTIFICATION ELAPSED SINCE SEEDING?
YES
NO
405
BINARIZATION PROCESS
406
EROSION/DILATION PROCESS
407
LABELING PROCESS
408
SORT WHITE PIXEL MASSES BY THE NUMBER OF PIXELS
409
LARGEST WHITE PIXEL MASS SIZE EQUAL TO OR GREATER THAN THRESHOLD VALUE?
NO
YES
A
...[CONTINUED TO FIG. 10
410
SET ARBITRARY COORDINATES AT MAGNIFYING OBSERVATION POSITION
PICK UP MAGNIFIED IMAGE
402
END

FIG. 9

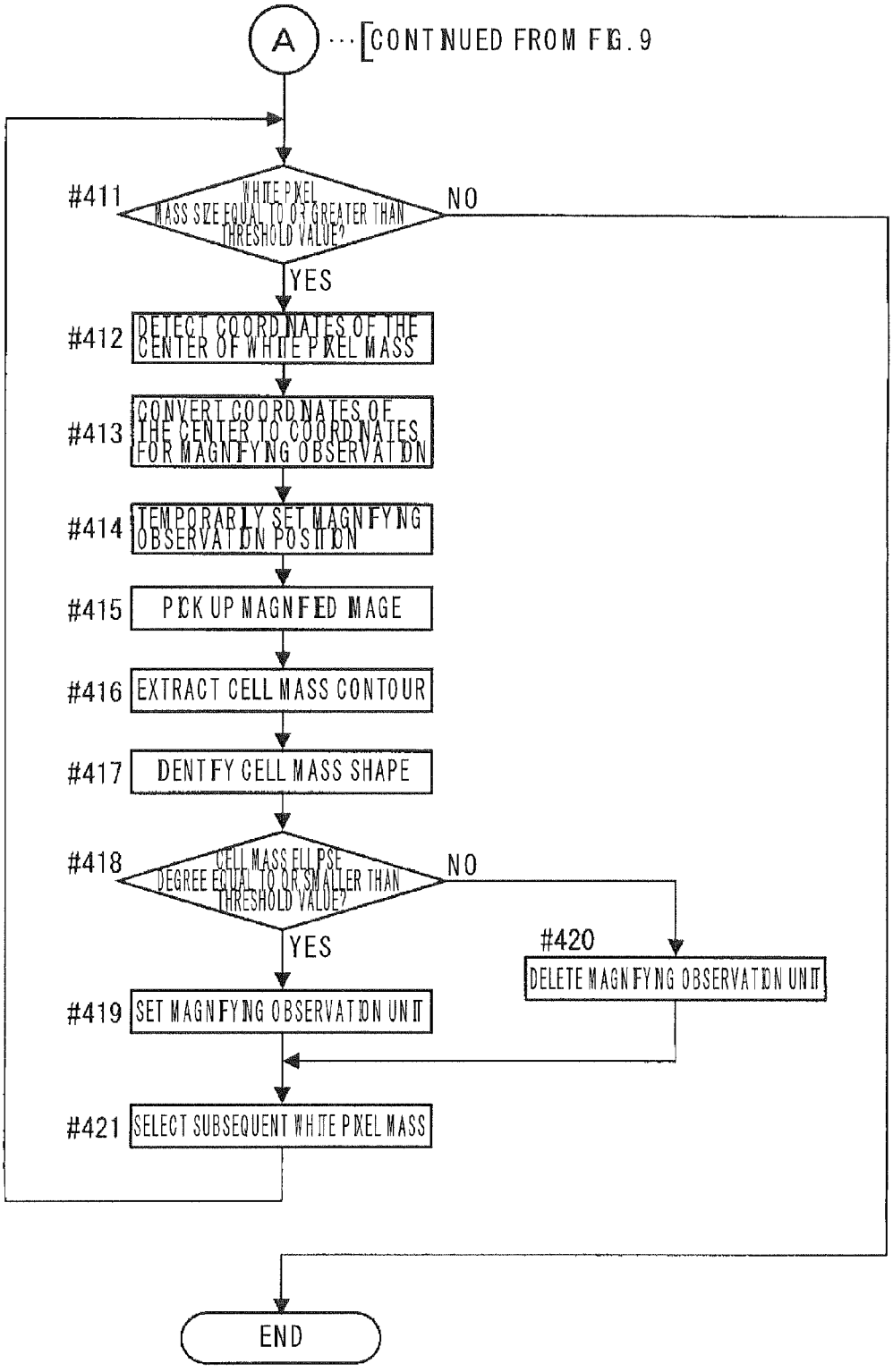
A
...[CONTINUED FROM FIG. 9
#411
WHITE PIXEL MASS SIZE EQUAL TO OR GREATER THAN THRESHOLD VALUE?
NO
YES
#412
DETECT COORDINATES OF THE CENTER OF WHITE PIXEL MASS
#413
CONVERT COORDINATES OF THE CENTER TO COORDINATES FOR MAGNIFYING OBSERVATION
#414
TEMPORARILY SET MAGNIFYING OBSERVATION POSITION
#415
PICK UP MAGNIFIED IMAGE
#416
EXTRACT CELL MASS CONTOUR
#417
IDENTIFY CELL MASS SHAPE
#418
CELL MASS ELLIPSE DEGREE EQUAL TO OR SMALLER THAN THRESHOLD VALUE?
NO
YES
#420
DELETE MAGNIFYING OBSERVATION UNIT
#419
SET MAGNIFYING OBSERVATION UNIT
#421
SELECT SUBSEQUENT WHITE PIXEL MASS
END

FIG. 10

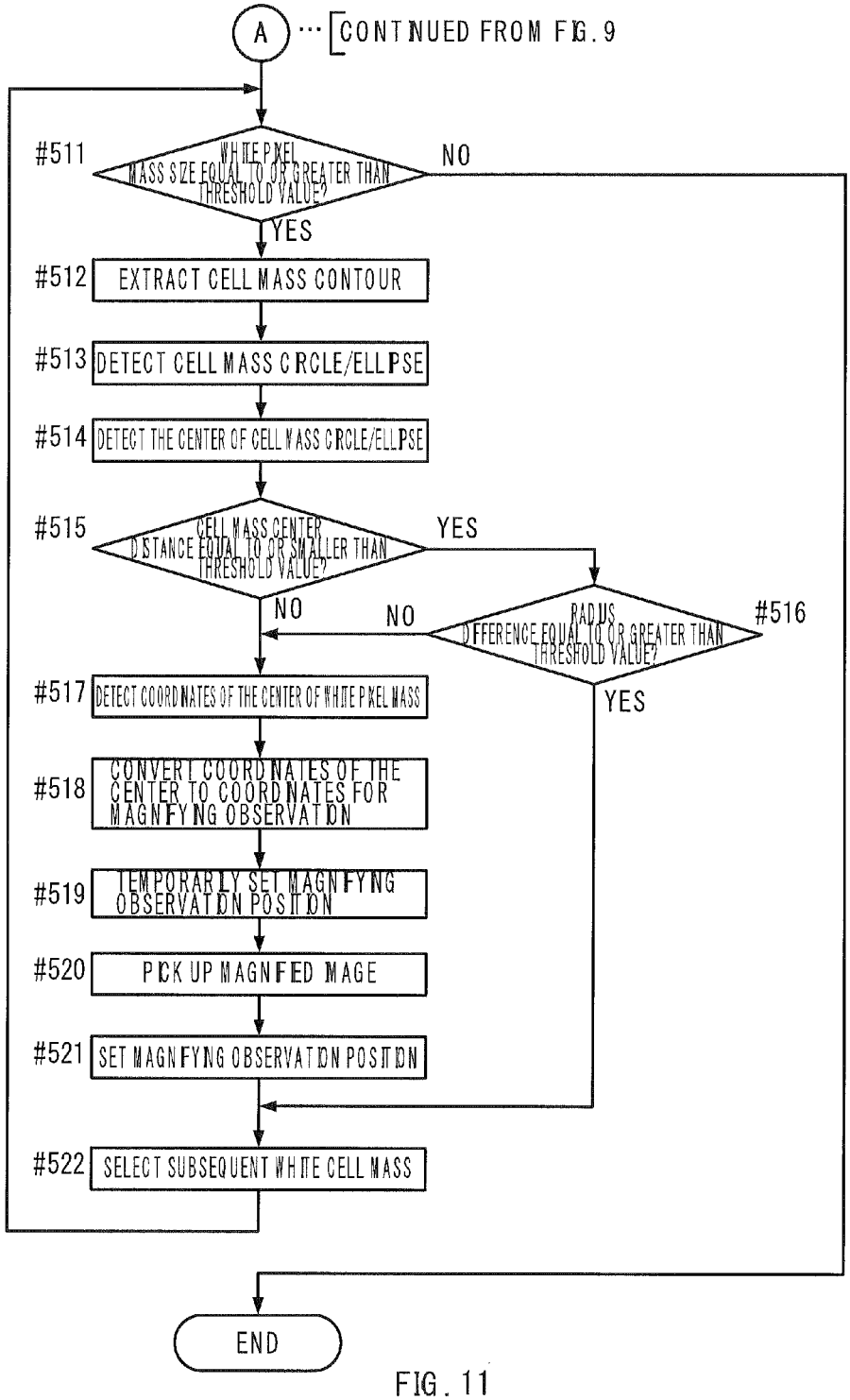
A
CONTINUED FROM FIG. 9
#511
WHITE PIXEL MASS SIZE EQUAL TO OR GREATER THAN THRESHOLD VALUE?
NO
YES
#512
EXTRACT CELL MASS CONTOUR
#513
DETECT CELL MASS CIRCLE/ELLIPSE
#514
DETECT THE CENTER OF CELL MASS CIRCLE/ELLIPSE
#515
CELL MASS CENTER DISTANCE EQUAL TO OR SMALLER THAN THRESHOLD VALUE?
YES
NO
#516
RADIUS DIFFERENCE EQUAL TO OR GREATER THAN THRESHOLD VALUE?
NO
YES
#517
DETECT COORDINATES OF THE CENTER OF WHITE PIXEL MASS
#518
CONVERT COORDINATES OF THE CENTER TO COORDINATES FOR MAGNIFYING OBSERVATION
#519
TEMPORARILY SET MAGNIFYING OBSERVATION POSITION
#520
PICK UP MAGNIFIED IMAGE
#521
SET MAGNIFYING OBSERVATION POSITION
#522
SELECT SUBSEQUENT WHITE CELL MASS
END

FIG. 11

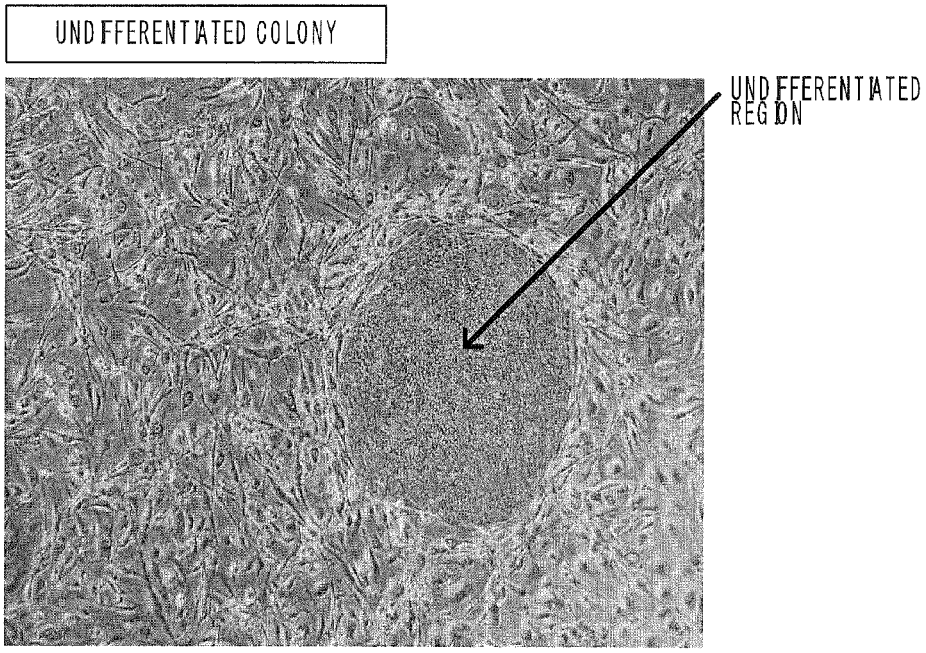
UNDIFFERENTIATED COLONY
UNDIFFERENTIATED REGION

FIG. 12

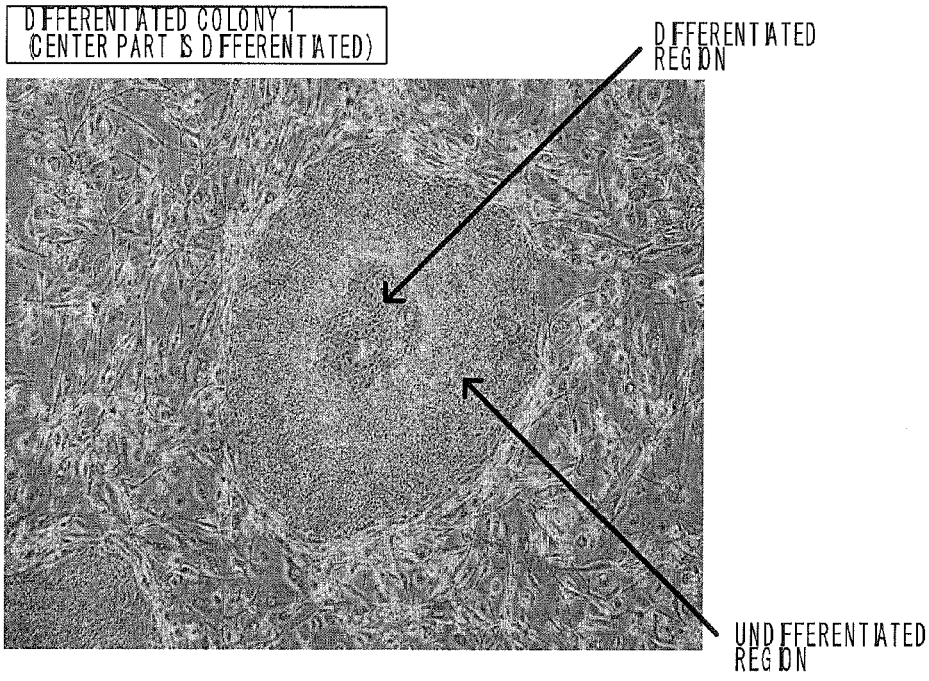
DIFFERENTIATED COLONY 1
(CENTER PART IS DIFFERENTIATED)
DIFFERENTIATED REGION
UNDIFFERENTIATED REGION

FIG. 13

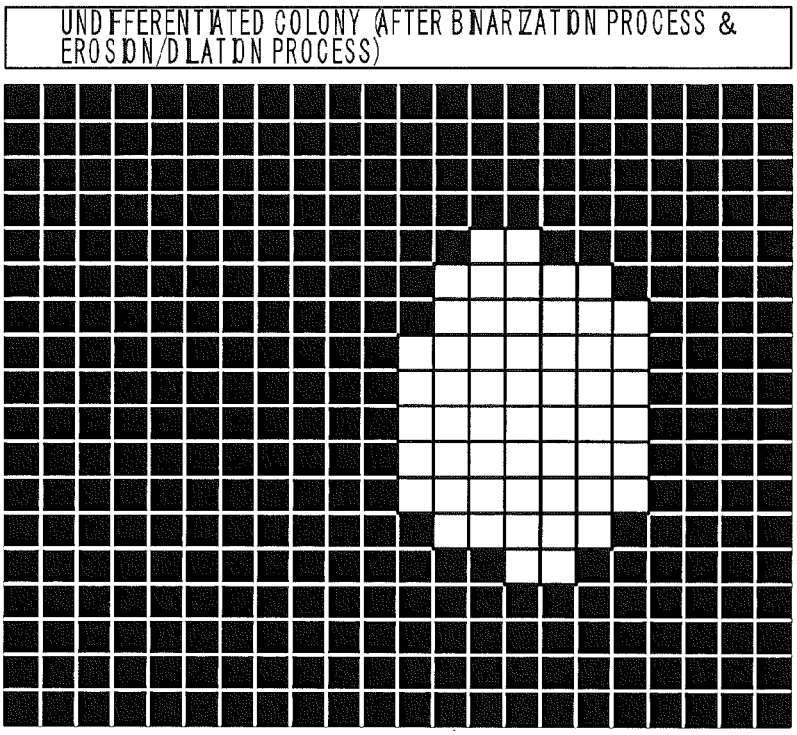
UNDIFFERENTIATED COLONY (AFTER BINARIZATION PROCESS & EROSION/DILATION PROCESS)

FIG. 14

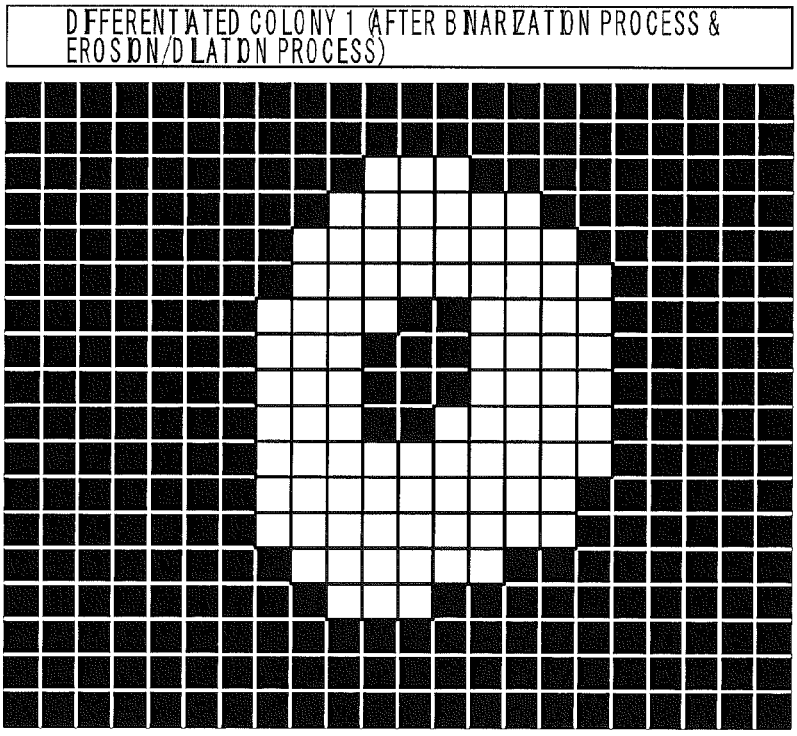
DIFFERENTIATED COLONY 1 (AFTER BINARIZATION PROCESS & EROSION/DILATION PROCESS)

FIG. 15

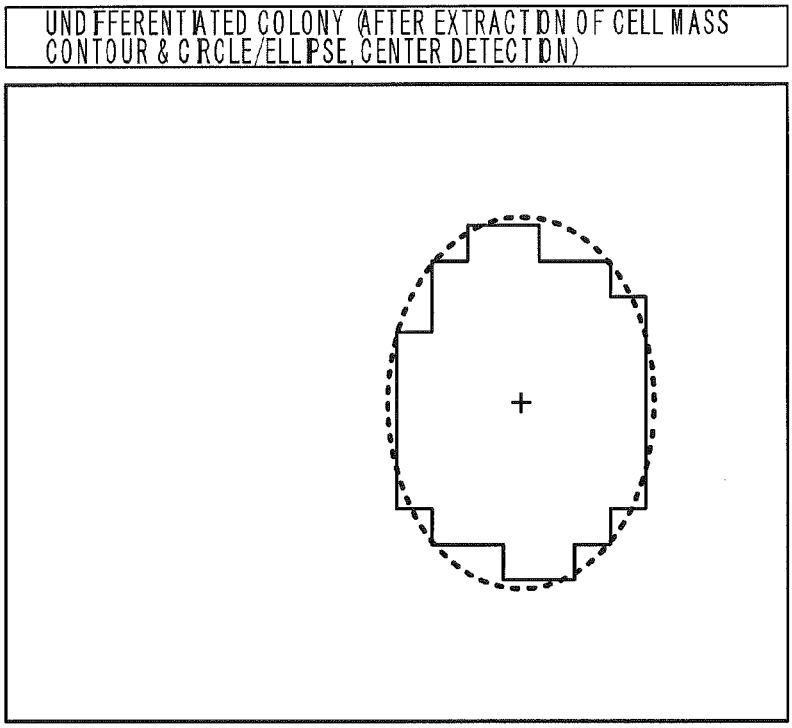
UNDIFFERENTIATED COLONY (AFTER EXTRACTION OF CELL MASS CONTOUR & CIRCLE/ELLIPSE, CENTER DETECTION)

FIG. 16

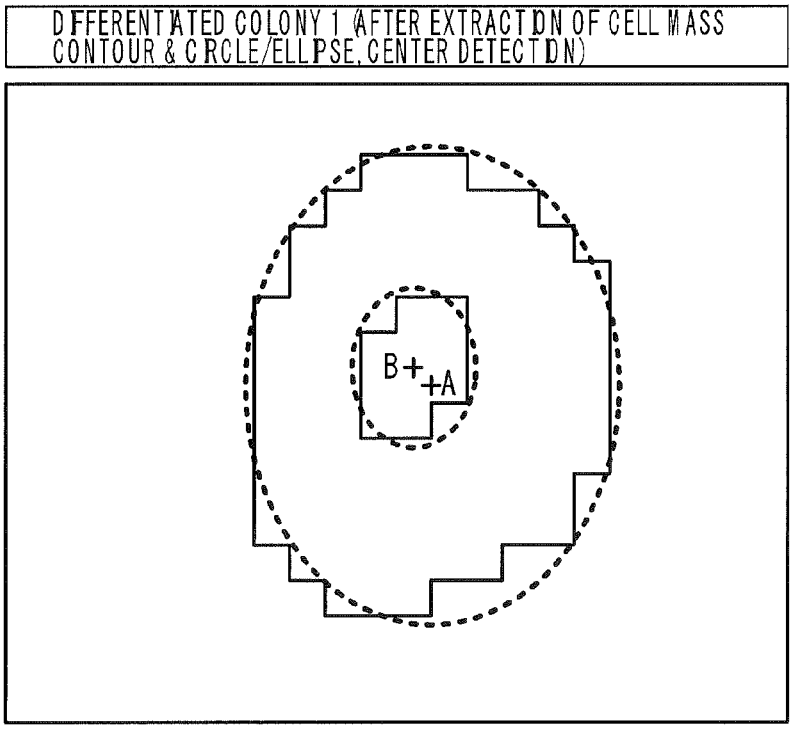
DIFFERENTIATED COLONY 1 (AFTER EXTRACTION OF CELL MASS CONTOUR & CIRCLE/ELLIPSE, CENTER DETECTION)
B+
+A

FIG. 17

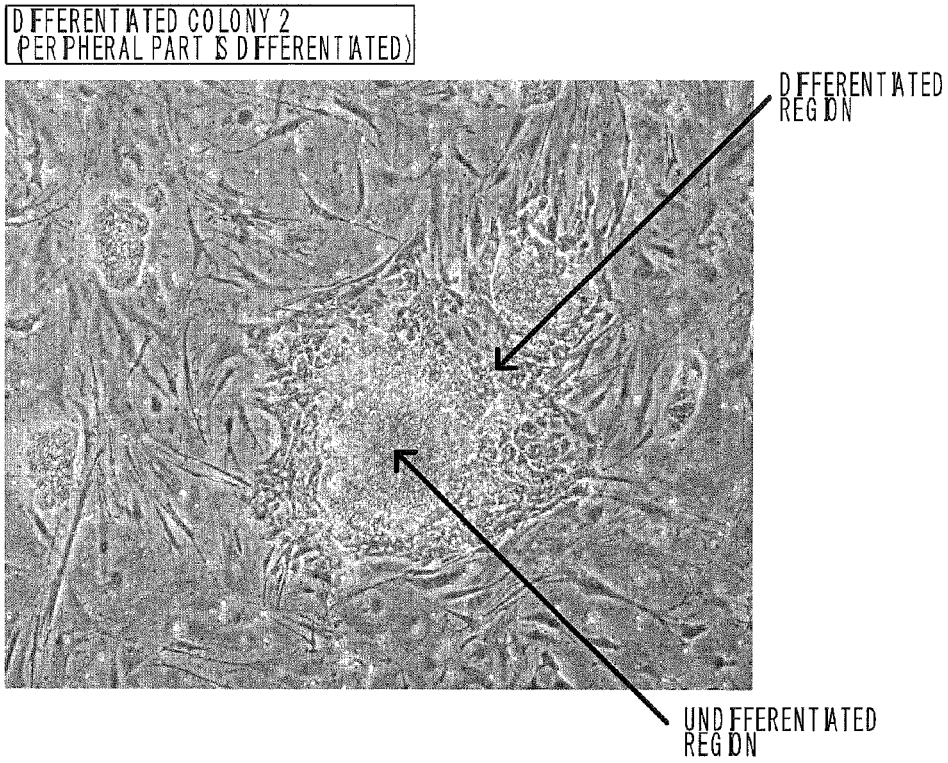
DIFFERENTIATED COLONY 2
(PERIPHERAL PART IS DIFFERENTIATED)
DIFFERENTIATED REGION
UNDIFFERENTIATED REGION

FIG. 18

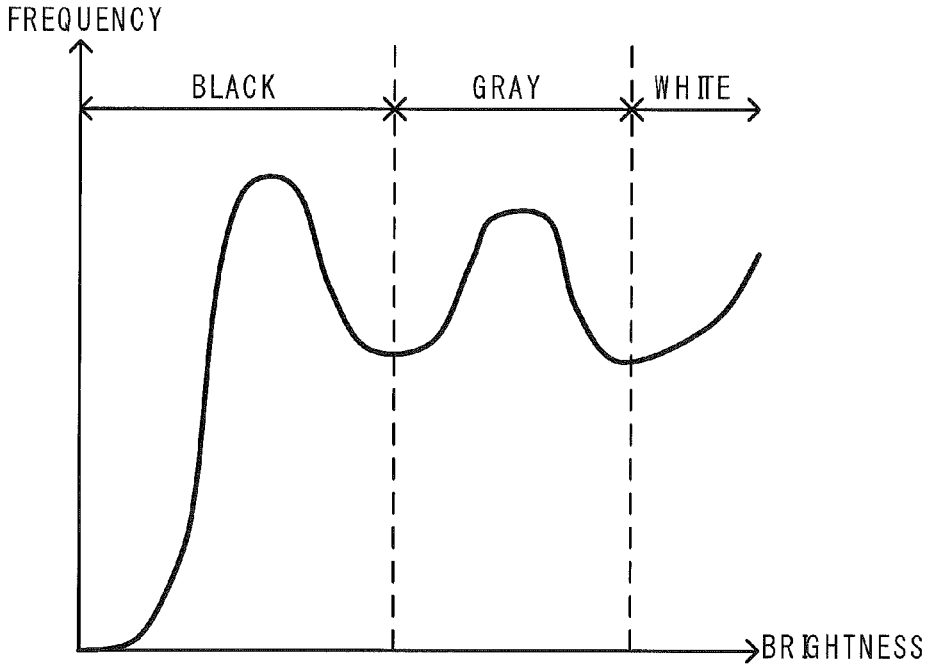
FREQUENCY
BLACK
GRAY
WHITE
BRIGHTNESS

FIG. 19

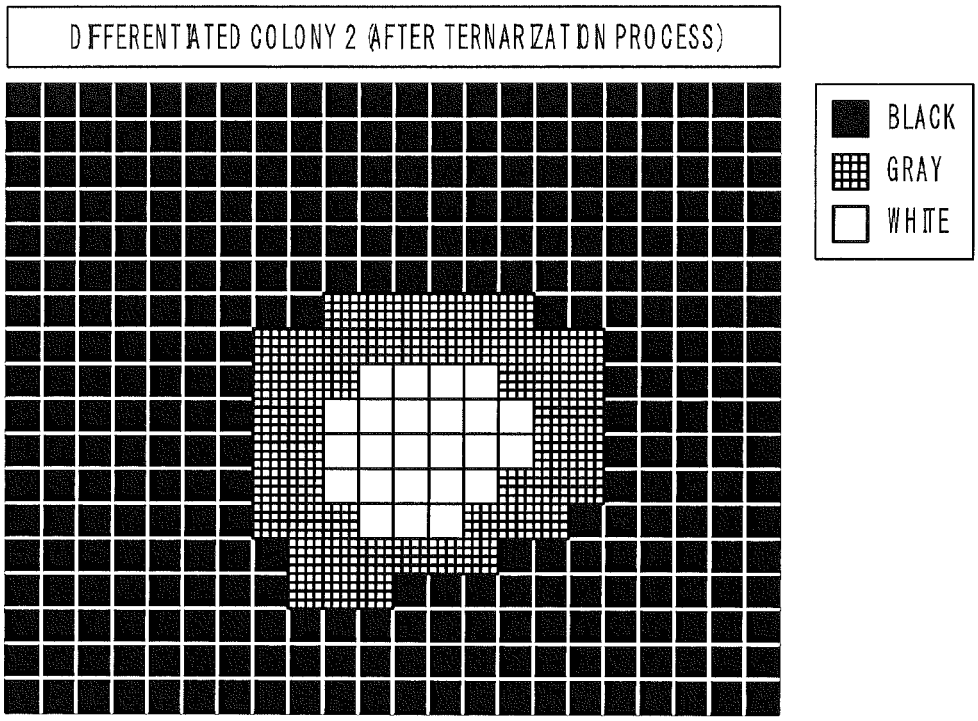
DIFFERENTIATED COLONY 2 (AFTER TERNARIZATION PROCESS)
BLACK
GRAY
WHITE

FIG. 20

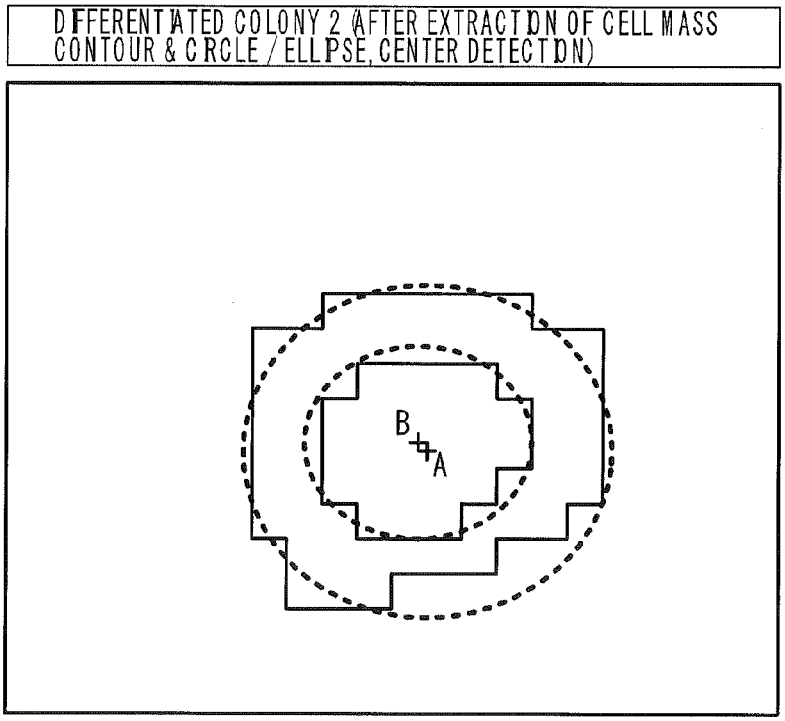
DIFFERENTIATED COLONY 2 (AFTER EXTRACTION OF CELL MASS CONTOUR & CIRCLE / ELLIPSE, CENTER DETECTION)
B
A

FIG. 21

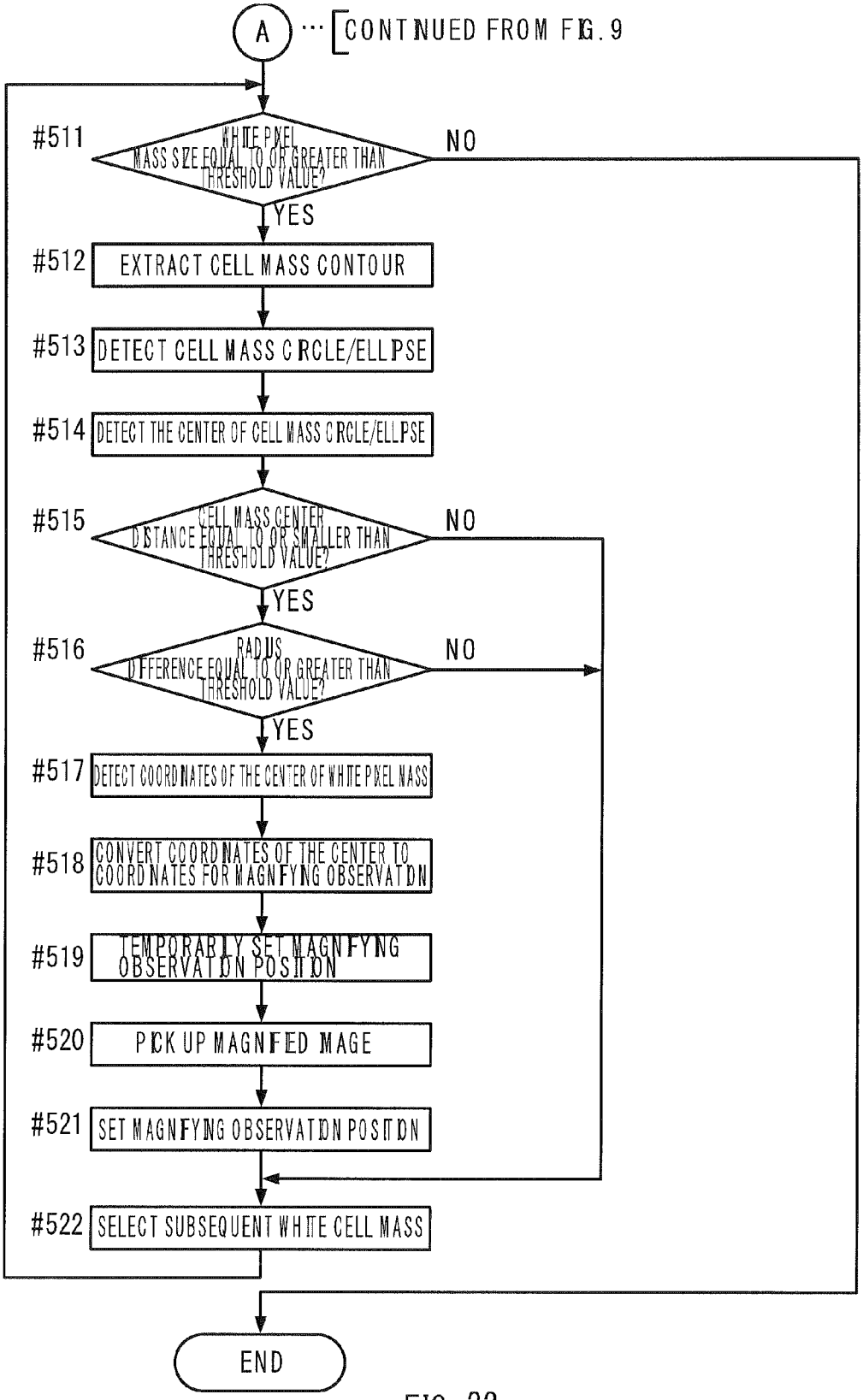
A
CONTINUED FROM FIG. 9
#511
WHITE PIXEL MASS SIZE EQUAL TO OR GREATER THAN THRESHOLD VALUE?
NO
YES
#512
EXTRACT CELL MASS CONTOUR
#513
DETECT CELL MASS CIRCLE/ELLIPSE
#514
DETECT THE CENTER OF CELL MASS CIRCLE/ELLIPSE
#515
CELL MASS CENTER DISTANCE EQUAL TO OR SMALLER THAN THRESHOLD VALUE?
NO
YES
#516
RADIUS DIFFERENCE EQUAL TO OR GREATER THAN THRESHOLD VALUE?
NO
YES
#517
DETECT COORDINATES OF THE CENTER OF WHITE PIXEL MASS
#518
CONVERT COORDINATES OF THE CENTER TO COORDINATES FOR MAGNIFYING OBSERVATION
#519
TEMPORARILY SET MAGNIFYING OBSERVATION POSITION
#520
PICK UP MAGNIFIED IMAGE
#521
SET MAGNIFYING OBSERVATION POSITION
#522
SELECT SUBSEQUENT WHITE CELL MASS
END

FIG. 22

RECORDING MEDIUM HAVING OBSERVATION PROGRAM RECORDED THEREIN AND OBSERVATION APPARATUS

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation under 35 U.S.C. §120 of PCT/JP2011/079763, filed Dec. 22, 2011, which is incorporated herein reference and which claimed priority to Japanese Application No. 2011-042418, filed Feb. 28, 2011. The present application likewise claims priority under 35 U.S.C. §119 to Japanese Application No. 2011-042418, filed Feb. 28, 2011, the entire content of which is also incorporated herein by reference.

BACKGROUND

Description of the Related Art

In culturing cells, if observation can be started concurrently with emergence of a cell mass having a plurality of cells gathering therein and the observation can be conducted sequentially in chronological order, it can be a promising technique to support regenerative medicine, for example. Conventionally, such observation of cells has been conducted, by using a microscope, etc., when replenishment or replacement of culture fluid in a culture container is required during cell culture, and an image has been picked up as necessary.

However, observation of cells using a microscope requires much expense in time and effort. For example, in order to identify a cell mass having emerged in a container, first, the entire container needs to be observed visually or by using a microscope, and then, a growing state of the individual cell mass needs to be observed under magnification by replacing an objective lens. In magnifying observation, a narrow field of view causes difficulty in searching a target cell mass, and also difficulty in matching the cell mass with the field of view. When observing cells, it is preferable to conduct time-lapse observation in which a long-term change is observed from a time of emergence of the cell mass to a time of completion of growth thereof in every predetermined time period. Since the cell mass cannot be observed visually or by using a low-magnification microscope immediately after seeding of the cell, an observation position needs to be searched and set again several days later.

Further, in conventional observation that is conducted at the time of replenishment or replacement of culture fluid in a culture container usually once in one to three days, it is difficult to conduct observation from a time of emergence of a cell mass, and thus a technique capable of observation of a cell mass from the time of emergence thereof is in high demand. Further, in picking up an image of a cell in each of the cases where the entire container is observed and where a part of the interior of the container is observed under magnification, such a problem arises that heat generated from illumination, a lens driving system has effects on growth of the cell.

With respect to such observation of cells, a device has been proposed that is configured to save time and effort in switching between the observation of the entire container and the magnifying observation of a part of the interior of the container, and an example can be found in Japanese Laid-Open Patent Publication No. 2009-198709. The observation apparatus described in Japanese Laid-Open Patent Publication No. 2009-198709 includes at least two image-pickup optical systems having different magnifications for picking up images of an object to be observed, and a reference value of a low-magnification image is calculated such that the image feature of an image picked up by a high-magnification image-pickup optical system becomes substantially equal to the image feature of an image concurrently picked up in a low-magnification image-pickup optical system.

However, since the high-magnification image-pickup optical system and the low-magnification image-pickup optical system pick up images by using a common light source in the observation apparatus described in Japanese Laid-Open Patent Publication No. 2009-198709, a cell in the container cannot be observed in detail. That is because the high-magnification image-pickup optical system and the low-magnification image-pickup optical system require different types of illumination, respectively, that is, the high-magnification image-pickup optical system needs illumination such as a point source and a ring slit used in a phase-difference optical system suitable for observation of a substantially transparent cell in a micro region, while the low-magnification image-pickup optical system needs illumination such as planar light source suitable for observation of a relatively wide field of view. In other words, although distribution of cells in the container can be observed, it is concerned that identification of a cell mass having emerged in the container might fail or a target cell mass might be erroneously identified. Further, continuous observation from a time of emergence of a cell mass to a time of completion of growth thereof is not considered, either.

At least some present embodiments were made in view of the above points to provide an observation apparatus capable of identification of a sample mass having emerged through observation of the entire container and detailed observation of the identified sample mass by magnification thereof, in observing the sample such as a cell in the container. Further, at least some embodiments may provide an observation program and an observation system capable of continuous observation of such identified sample mass from a time of emergence thereof to a time of completion of growth thereof.

SUMMARY

A recording medium having an observation program recorded therein according to an aspect of the present invention, the observation program configured to cause a computer to execute: an entire image-pickup process of picking up an image of a sample by picking up an image of an entire container containing the sample and a solution; a sample mass identification process of identifying a sample mass having a plurality of the samples gathering therein, from the image picked up in the entire image-pickup process; a sample mass determination process of extracting shape information of the sample mass identified in the sample mass identification process, and determining a state of the sample mass based on the shape information; a coordinate detection process of selecting a magnifying observation target sample mass from the sample masses identified in the sample mass identification process, and detecting coordinates of a center of the magnifying observation target sample mass, based on a determination result of the sample mass determination process; and a magnifying image-pickup process of performing magnification with the coordinates detected in the coordinate detection process as a center of the magnification, and picking up an image of the magnifying observation target sample mass.

Other features will become apparent from descriptions of this specification and of the accompanying drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

For more thorough understanding, the following description should be read in conjunction with the accompanying drawings, in which:

FIG. **1** is a configuration diagram of an observation system according to a first embodiment;

FIG. **2** is a perpendicular sectional side view of an observation apparatus of an observation system illustrated in FIG. **1**;

FIG. **3** is a perpendicular sectional front view of an observation apparatus of an observation system illustrated in FIG. **1**;

FIG. **4** is a perpendicular sectional side view of an observation apparatus similar to FIG. **2** and illustrates a state where a container has been moved to a spot corresponding to an entire observation unit;

FIG. **5** is a block diagram illustrating a configuration of a computer of an observation system illustrated in FIG. **1**;

FIG. **6** is an explanatory diagram illustrating a flow according to an operation of an observation system in FIG. **1**;

FIG. **7** is a flowchart illustrating an operation according to an observation process in an observation system in FIG. **1**;

FIG. **8** is a flowchart illustrating an operation according to an observation process in an observation program according to a second embodiment;

FIG. **9** is a flowchart illustrating an operation according to an observation process in an observation program according to a third embodiment;

FIG. **10** is a continuation of a flowchart illustrating an operation according to an observation process illustrated in FIG. **9**;

FIG. **11** is a flowchart illustrating an operation according to an observation process in an observation program according to a fourth embodiment;

FIG. **12** is a diagram illustrating an undifferentiated colony as an example of a cell mass having an appropriate shape for continuing observation;

FIG. **13** is a diagram illustrating a differentiated colony whose center part is differentiated as an example of a cell mass having a shape inappropriate for continuing observation;

FIG. **14** is a diagram illustrating an example of a result obtained by applying a binarization process and an erosion/dilation process to an image of an undifferentiated colony;

FIG. **15** is a diagram illustrating an example of a result obtained by applying a binarization process and an erosion/dilation process to an image of a differentiated colony whose center part is differentiated;

FIG. **16** is a diagram illustrating an example of a result obtained by further applying contour extraction, circle/ellipse detection, and circle/ellipse center detection to an image of an undifferentiated colony;

FIG. **17** is a diagram illustrating an example of a result obtained by further applying contour extraction, circle/ellipse detection, and circle/ellipse center detection to an image of an undifferentiated colony;

FIG. **18** is a diagram illustrating a differentiated colony whose peripheral part is differentiated as an example of a cell mass having a shape not appropriate for continuing observation;

FIG. **19** is a histogram illustrating an example of frequency distribution of brightness of each pixel in an image of a differentiated colony whose peripheral part is differentiated;

FIG. **20** is a diagram illustrating an example of a result obtained by applying a ternarization process to an image of a differentiated colony whose peripheral part is differentiated;

FIG. **21** is a diagram illustrating an example of a result obtained by further applying contour extraction, circle/ellipse detection, and circle/ellipse center detection to an image of a differentiated colony whose peripheral part is differentiated; and

FIG. **22** is a flowchart illustrating an operation according to an observation process in an observation program targeting to a differentiated colony in magnifying observation.

DETAILED DESCRIPTION OF THE INVENTION

At least the following details will become apparent from descriptions of this specification and of the accompanying drawings.

In the following description, a "predetermined size" indicates a size of a sample mass set in advance and of the order of such a size that can be determined to be observed under magnification, and may be defined by the number of pixels on an image, for example. For example, if an image of a field of view with 40 mm×40 mm is picked up by a camera having 5 million pixels in entire observation, approximately 1000 pixels are appropriate as predetermined size. In embodiments of the present invention which will be described later, the "predetermined size" is set at "1000 pixels" for the number of pixels, but it is not limited thereto. The same also applies to the "predetermined size" used in the following means.

A "predetermined identification time period" (predetermined time period relating to identification of a sample mass) indicates a time period set in advance relating to timing for making identification of a sample mass to be observed under magnification target. It indicates a time period of the order of such a time period in which a time of emergence of the sample mass can be determined, and the time period can be set at from several hours to several days, for example, but can be appropriately set as necessary. Therefore, in embodiments of the present invention which will be described below, the "predetermined time period relating to identification of a sample mass" is set at "1 day" but it is not limited thereto. The wording "predetermined time period" used in other means does not necessarily indicate a time period relating to timing for making identification of the sample mass to be observed under magnification or does not indicate the same period in terms of time.

"The predetermined number of days for identification" (the predetermined number of days) is the number of days set in advance relating to timing for ending identification of the sample mass to be observed under magnification, and may be set at the arbitrary number of days such as 5, 7 or 10 days. In embodiments of the present invention which will be described later, "the predetermined number of days" is set at "5 days" but it is not limited thereto.

A "predetermined image-pickup period" (predetermined time period relating to image pickup of a magnified image) indicates a time period set in advance relating to timing for picking up an image of a magnified sample mass and of the order of such a period during which a growing process of the sample mass can be grasped. It can be set at a time period of from several hours to several days, for example, but can be set as appropriate as necessary. Therefore, in embodiments of the present invention which will be described below, the "predetermined time period relating to image pickup of a magnified image" is set at "1 day" but it is not limited thereto. The wording "predetermined time period" used in other means does not necessarily indicate a time period relating to timing

for picking up an image of the magnified sample mass or does not necessarily represent the same time period in terms of time.

A "predetermined observation deadline" (predetermined deadline) is a deadline set in advance relating to timing for ending observation of a sample and may be set at an arbitrary deadline such as 10 days, 20 days or 30 days from the start of observation of the sample, for example. In embodiments of the present invention which will be described below, the "predetermined deadline" is set at "10 days", but it is not limited thereto.

A "predetermined shape" is a shape set in advance for a sample mass, and is preferably of the order of such a shape that can be determined to have a high possibility of continuously growing in such a manner as to be suitable for observation, is a circular shape to the highest degree possible, and can be set at an arbitrary numerical value such as an ellipse degree, for example. In embodiments of the present invention which will be described below, the "predetermined shape" is set at "an ellipse degree equal to or smaller than 1.1", but it is not limited thereto.

Hereinafter, embodiments of the present invention will be described on the basis of FIGS. **1** to **21**. Here, a cell in samples such as a cell, a germ, a microorganism is described as a sample, and a culture fluid is described as a solution, for example. Further, a cell mass having a plurality of cells gathering therein is described as a sample mass.

<First Embodiment>

First, regarding an observation system according to a first embodiment of the present invention, a configuration thereof will be described using FIGS. **1** to **5**. FIG. **1** is a configuration diagram of the observation system, FIG. **2** is a perpendicular sectional side view of an observation apparatus of the observation system, FIG. **3** is a perpendicular sectional front view of the observation apparatus, FIG. **4** is a perpendicular sectional side view of the observation apparatus illustrating a state where a container has been moved to a place corresponding to an entire observation unit, and FIG. **5** is a block diagram illustrating a configuration of a computer of the observation system. In the following description, it is assumed that the x-axis direction in FIGS. **2** and **3** is a left-and-right direction, the y-axis direction is a fore-and-aft direction (+y direction to the fore and −y direction to the aft), and the z-axis direction is an up-and-down direction.

The observation system S includes an observation apparatus **1**, a control device **100**, and a computer **200** as illustrated in FIG. **1**.

The observation apparatus **1** is a device configured to observe a sample such as a cell and connected to the control device **100**. The control device **100** is a device configured to control the observation apparatus **1**, and incorporates a driver, a controller, not shown, that is configured to drive the observation apparatus **1**. The control device **100** is connected to the computer **200**. The computer **200** is a so-called personal computer, for example, and executes an observation program, not shown, that is configured to observe the sample such as a cell. That is, the computer **200** is capable of sending an instruction to the control device **100**, to control the observation apparatus **1** and perform capture, storage of images picked up during observation.

The observation apparatus **1** includes an entire observation unit **10**, a magnifying observation unit **20**, a conveying unit **30**, and a driving unit **40** in a main body **2** that is a housing thereof as illustrated in FIGS. **1** to **4**.

The observation apparatus **1** is capable of observation using the entire observation unit **10** and the magnifying observation unit **20** of a cell in a container C containing the cell and a culture fluid of the cell that is arranged at the front center part of the device. The conveying unit **30** firmly holding the container C can be moved in a desired direction such as fore-and-aft or let-and-right direction and to a desired position by the driving unit **40**. The main body **2** is supported by leg portions **3** provided at 4 places to a floor surface. In the container C, a lid is provided so as to prevent contamination from the exterior of the container or contamination between itself and another container.

The entire observation unit **10** is provided at a portion on the front side of the interior of the sealed housing of the main body **2**, and includes a lens **11** which is an entire observation optical system, a CMOS camera **12** which is an image pickup unit, and a ring illumination **13** which is an entire observation illumination.

The lens **11** is arranged above a movement space of the conveying unit **30** firmly holding the container C so as to be able to observe downward the container C. The CMOS camera **12** is provided vertically above the lens **11**, and is arranged so that an image pickup element surface thereof is directed to the lens **11** provided below.

The ring illumination **13** has such a configuration that a plurality of LEDs attached so as to be directed diagonally upward are aligned in a ring shape and is arranged below the movement space of the conveying unit **30**. A space D is provided with a predetermined distance between the ring illumination **13** and the container C of the conveying unit **30** (See FIG. **4**). As a result, since a space in which air circulates is created between the ring illumination **13** and the container C, it becomes difficult to transfer heat generated by the ring illumination **13** to the container C, thereby being able to suppress an influence of heat generation caused by the ring illumination **13** upon growth of the cell. The ring illumination **13** applies light diagonally above, i.e., toward the center of the ring, and illuminates the cell in the container C as an observation target of the conveying unit **30** located above the ring illumination **13**. The CMOS camera **12** and the lens **11** are arranged so that the optical axes thereof match each other, and the ring illumination **13** is arranged so that the optical axis passes through the center of the ring illumination **13**.

With such a configuration, an image obtained via the lens **11** by irradiating the container C with light using the ring illumination **13** is formed on the image pickup element surface of the CMOS camera **12** in the entire observation unit **10**, and by picking up an image of the entire container C, an image of the cell in the container C is picked up. Then, the picked up image is stored, thereby facilitating identification and specification of a cell mass having a plurality of cells gathering therein in the container C.

Moreover, since the entire observation unit **10** applies light diagonally above to the container C from below the container C, the light passing through a spot where the cell is present in the bottom surface of the container C is scattered by the cell, and thus a part of the scattered light enters the camera, and the cell is seen white. The light passing through a spot where no cell is present is not scattered, and thus the light does not enter the camera and the cells is seen black. As described above, it is possible to apply appropriate light for specifying the cell emerging and growing in the vicinity of the inner bottom surface of the container C. Then, such a contrast can be obtained by which the external shape of the cell can be recognized as a white mass. Irradiation of the light from below produces such an effect of preventing that the observation becomes impossible due to blown-out highlights of the cell that are caused by light reflected by the lid of the container C.

The magnifying observation unit **20** is a so-called phase-contrast microscope, provided in the rear of the entire obser-

vation unit **10** in the interior of the sealed housing of the main body **2**, and includes: a magnifying observation optical system such as an objective lens **21**, a reflective mirror **22**, and a zoom lens **23**; a CCD camera **24** which is an image pickup unit; and a phase-contrast illumination unit **25** which is a magnifying observation illumination.

The objective lens **21** is arranged immediately below the movement space of the conveying unit **30**, and is provided so as to be capable of observing upward the interior of the container C. In the periphery of the objective lens **21** that is a lens unit closest to the bottom surface of the container C, an objective lens cover **26** is provided that is a cover member configured to prevent the heat generated in the lower part of the main body **2** from influencing the container C. Further, a window unit **27** is provided in an end of the upper part of the objective lens cover **26** between the objective lens **21** and the container C.

Here, the heat is generated from a motor, the camera, and the illumination in the sealed housing and filled therein, and also stays in the vicinity of the objective lens **21** and tries to radiate upward. If a cover configured to cover the entire magnifying observation optical system is used instead of the objective lens cover **26** having a small area, the proximity of the magnifying observation optical system and the bottom surface of the container C facilitates the transference of the heat in the housing, thereby facilitating increase in the temperature of the culture fluid.

In contrast thereto, in the objective lens cover **26**, the area of a place close to the bottom surface of the container C is made as small as possible, thereby suppressing the influence of the heat due to no air-circulation that is caused by very little space provided between the bottom surface of the container C and the objective lens cover **26**. At the same time, the objective lens cover **26** covers only the periphery of the objective lens **21**, thereby being able to increase the surface area of the objective lens cover **26**, and thus the heat can be scattered also in the lateral direction of the objective lens cover **26** in which the air can easily circulate, thereby being able to suppress heat transfer to the container C.

As such, by configuring the objective lens cover **26** provided, with the window unit **27** that covers only the periphery of the objective lens **21**, between the magnifying observation optical system and the container C, it becomes difficult to transfer the heat generated by the magnifying observation optical system to the container C. Thus, it can be suppressed that the influence of the heat generation of the lens driving system is exerted upon the growth of the cell.

The reflective mirror **22** is arranged below the objective lens **21**, and provided with such an inclination as to reflect light substantially horizontally backward. The reflective mirror **22** guides an image obtained from the objective lens **21** to the zoom lens **23** at the rear. The zoom lens **23** is arranged in such a manner as to extend in the fore-and-aft direction to the rear of the reflective mirror **22**, and is configured to magnify the image obtained from the objective lens **21**. The CCD camera **24** is provided in the further rear of the zoom lens **23**, and is arranged such that an image pickup element surface thereof is directed toward the zoom lens **23** in the front.

The phase-contrast illumination unit **25** is provided in the upper part of the main body **2**, and includes an LED **25***a* and a reflective mirror **25***b*. The LED **25***a* irradiates, with light, the cell to be observed in the container C of the conveying unit **30** located below the phase-contrast illumination unit **25**. The reflective mirror **25***b* is arranged vertically above the objective lens **21**, and is configured to reflect light so that the light applied by the LED **25***a* reaches the objective lens **21** via the container C.

With such a configuration, the magnifying observation unit **20** irradiates the container C, with light, by using the phase-contrast illumination unit **25**, thereby forming an image obtained via the objective lens **21**, the reflective mirror **22**, and the zoom lens **23** on the image-pickup element surface of the CCD camera **24**, and the area of a part in the container C is magnified, to pick up an image of the cell in the container C. Then, the picked-up image is stored, thereby facilitating identification and specification of a cell mass in the container C and close observation thereof.

In the magnifying observation unit **20**, the relatively heavy magnifying observation optical system, including a plurality of the lenses and the zoom mechanisms thereof to magnify and observe the cell, is arranged in the lower part, which leads to an appropriate weight balance of the device, thereby being able to make stable magnifying observation. Further, since the objective lens **21** can be brought closer from below the container C toward the cell that emerges and grows in the vicinity of the inner bottom surface of the container C, the cell can be observed at relatively great magnification by reducing a focal distance. Further, the observation is made from below the container C in the magnifying observation unit **20**, which enables observation without being affected by stains on the lid of the container C.

The conveying unit **30** is provided at the front center part of the main body **2**, in such a manner as to be sandwiched by the ring illumination **13** of the entire observation unit **10** as well as the magnified observation optical system of the magnifying observation unit **20**, that are disposed bellow; and the entire observation optical system of the entire observation unit **10** as well as the phase-contrast illumination unit **25** of the magnifying observation unit **20**, that are disposed above. The conveying unit **30** includes a holder **31**, and this holder **31** grasps the container C containing the cell to be observed and the culture fluid for the cell. The holder **31** is positioned with respect to the entire observation unit **10** and the magnifying observation unit **20**, and the container C is positioned with respect to the holder **31**. As a result, even if the container C and the holder **31** are removed together and the culture fluid is replaced or a reagent is charged, the same spot can be easily observed both in the entire observation unit **10** and the magnifying observation unit **20**.

The driving unit **40** is provided in the rear and on the side of the conveying unit **30**, and includes an x-axis driving mechanism **41**, an x-axis motor **42**, a y-axis driving mechanism **43**, a y-axis motor **44**, a z-axis motor **45**, and a zoom motor **46**. As illustrated in FIGS. **2** and **3**, a description will be made assuming that the left-and-right direction with respect to the observation apparatus **1** is the x-axis, the fore-and-aft direction is the y-axis, and the up-and-down direction is the z-axis.

The x-axis driving mechanism **41** is arranged immediately rear of the conveying unit **30** as well as directly supports the conveying unit **30**. The x-axis driving mechanism **41** includes a belt, a pulley, a slide guide member, a shaft, not shown; is driven by the x-axis motor **42**; and moves the conveying unit **30** in the left-and-right direction. The y-axis driving mechanism **43** is arranged in a place on the side surface of the conveying unit **30** and the main body **2**, and supports the x-axis driving mechanism **41**. The y-axis driving mechanism **43** includes a belt, a pulley, a slide guide member, not shown; is driven by the y-axis motor **44**; and moves the conveying unit **30** in the fore-and-aft direction together with the x-axis driving mechanism **41** (See FIG. **4**).

By operating such driving mechanisms, the conveying unit **30** conveys the container C from the entire observation unit **10** to the magnifying observation unit **20** or in the opposite

direction. Since the container C is moved, even if the entire observation unit **10** and the magnifying observation unit **20** are arranged at places far from each other, it becomes possible to observe the entire container C and identify an emerging cell mass, and further, magnify and observe this identified cell mass in detail.

The conveying unit **30** is configured to convey the container C in a direction orthogonal to the optical axis direction of the entire observation unit **10** and the magnifying observation unit **20** as described above, and at least one direction in the conveying directions, that is, the fore-and-aft direction is made common therebetween, thereby matching coordinates in the observation field of view in the entire observation unit **10** with coordinates in the observation field of view in the magnifying observation unit **20**. Thus, the coordinates in the observation fields of view in the entire observation unit **10** and the magnifying observation unit **20** match each other, thereby being able to easily identify, using the magnifying observation unit **20**, the cell mass specified through the observation of the entire container C by the entire observation unit **10**. Therefore, it is prevented to erroneously identify a target cell mass, thereby being able to realize observation with high accuracy.

The z-axis motor **45** and the zoom motor **46** are arranged in the main body **2** in the rear of the conveying unit **30**. The z-axis motor **45** is a motor configured to move the magnified observation optical system and the CCD camera **24** in the up-and-down direction. The zoom motor **46** is a motor configured to change a magnification of the zoom lens **23**, and is capable of changing magnification of an image to be picked up.

The computer **200** includes at least an arithmetic unit **201** as illustrated in FIG. **5**. The computer **200** may also include a storage unit **210**, a time-measuring unit **202**, an input unit **203**, and an output unit **204**.

The arithmetic unit **201** is constituted by a common microcomputer and other electronic components, and is configured to control a series of observation operations relating to the observation apparatus **1** on the basis of an observation program **220** and other data, etc., that are stored/inputted in/to the microcomputer, the storage unit **210**, etc. An image process unit may be separately provided that is configured to execute processes of images picked up by the entire observation unit **10** or the magnifying observation unit **20**.

The observation program **220** executed in the arithmetic unit **201** includes a cell mass identification unit **221**, a cell mass sorting unit **222**, a coordinate detection unit **223**, a coordinate conversion unit **224**, a cell mass extraction unit **225**, a shape identification unit **226**, and a shape determination unit **227** as illustrated in a hardware manner in a functional block diagram in FIG. **5**. The observation program **220** executes: an entire image-pickup process of picking up an image of a cell, by sending an instruction to the entire observation unit **10** of the observation apparatus **1** and picking up an image of the entire container C; and a magnifying image-pickup process of picking up an image of a cell, by sending an instruction to the magnifying observation unit **20** and magnifying the interior of the container C, in addition to each of these process blocks.

The cell mass identification unit **221** first converts an image into a gray image if it is a color image, and then, discriminates a part that is not a cell mass and a part of a cell mass in the image picked up in the entire image-pickup process using a predetermined threshold value. As a result, binarization is executed so that the part that is not a cell mass is made into black and the cell mass part is made into white. Then, the cell, that is, the number of white pixels is calculated. A method of calculating the number of white pixels includes: a labeling method of calculating a connected region of white pixels; and a small-region method of calculating a region so that the number of white pixels in a small region, that is determined in advance at an arbitrary position, becomes as great as possible.

The labeling method is a method of identifying a cell mass by a size of a single white pixel region or the degree of density in the white pixel regions, while the small region method is a method of identifying a cell mass by the number of the white pixel regions, the range of the number thereof, and the degree of density thereof. In addition, identification may be made by the degree of isolation of the cell masses (the degree that individual cell masses exist with a predetermined distance therebetween). Here, the labeling method is employed.

The labeling process is a process of grouping a plurality of pixels by assigning the same number (label) to white pixels (or black pixels) adjacent to each other in the image subjected to the binarization process. In determination on adjacency in the labeling process, four-connection (four-neighbor) and eight-connection (eight-neighbor) are used. In the four-connection, if pixels continue up and down and right and left with respect to the pixel of interest, they are determined to be adjacent, while in the eight-connection, determination on adjacency is made considering continuation in diagonal four more directions. As such, the cell mass identification unit **221** identifies a mass of binarized white pixels, that is, a cell mass from an image picked up in the entire image-pickup process.

Then, the cell mass identification unit **221** is configured to recognize a cell mass having a size equal to or greater than a predetermined size in identified cell masses as a magnifying observation target. The "predetermined size" indicates a size set in advance for a cell mass, and of the order of such a size that can be determined to be observed under magnification. Here, the predetermined size is set at 1000 pixels for the number of pixels, for example, and is stored in the storage unit **210**. As a result, a cell mass having pixels equal to or more than 1000 pixels as the number of pixels is recognized as a magnifying observation target cell mass, thereby being able to determine a time of emergence of a cell mass. Therefore, it becomes possible to perform continuous observation from a time of emergence of a cell mass to a time of completion of growth thereof.

The cell mass sorting unit **222** is configured to execute sorting of the cell masses identified by the cell mass identification unit **221**, that is, masses of white pixels in order from that having the greater number of pixels. Then, the predetermined number of cell masses in order from that having the greater number of pixels, for example, is selected as observation targets.

The coordinate detection unit **223** is configured to detect coordinates of the center of a cell mass that is identified by the cell mass identification unit **221** and sorted by the cell mass sorting unit **222**, that is, the mass of white pixels.

The coordinate conversion unit **224** is configured to first calculate a coordinate by a pixel on an image picked up in the entire image-pickup process. Then, the coordinate is converted into a real scale with the center of an image as the origin. Here, various aberrations such as distortion aberration of the image may be corrected. Further, the coordinate conversion unit **224** is configured to convert the real scale into the motor pulse numbers of the x-axis motor **42** and the y-axis motor **44** of the driving unit **40** in the observation apparatus **1**, so as to be matched with a position on the image expressed by this real scale. As such, the coordinate conversion unit **224** is configured to form a common coordinate system in which the coordinate on the image picked up in the magnifying image-pickup process matches the coordinate on the image picked up in the entire image-pickup process.

The cell mass extraction unit **225** is configured to extract a cell mass at the coordinates detected by the coordinate detection unit **223** from the image picked up in the magnifying image-pickup process.

The shape identification unit **226** is configured to first perform matching of a patch image prepared in advance with an image picked up in the magnifying image-pickup process. As a matching result, range images, expressed by shading, of the image picked up in the magnifying image-pickup process and the patch image are obtained. Then, the shape identification unit **226** is configured to execute the binarization process for the range image using a predetermined threshold value. Matching methods includes a template matching, a histogram matching, for example, and an image to be determined, that is, an image picked up in the magnifying image-pickup process is subjected to raster scan by the patch image, thereby calculating ranges of the both. If a large number of patch images are prepared, the range images of the matching results are integrated. Even if a plurality of cell masses are present in the image picked up in the magnifying image-pickup process, the shape identification unit **226** can identify each of the cell masses separately.

Subsequently, the shape identification unit **226** detects a contour by executing contour extraction by an edge extraction filter and contour tracing by eight-connection search, for example, in an image subjected to the binarization process. As the edge extraction filter in contour extraction, a differential filter, a Prewitt filter, a Sobel filter, a Canny Edge Detector can be used, for example. In the contour tracing, a contour line can be extracted by tracing contour points sequentially in one direction from a tracing start point of a contour, and four-connection search can be also used.

Then, the shape identification unit **226** detects a predetermined shape such as a circle, an ellipse, a rectangle from the contour detection results. As a method of detecting a circle from a contour or an edge, Hough transform can be used. As a method of detecting an ellipse from a contour or an edge, a method of fitting an ellipse to a sequence of points of a contour by generalized Hough transform or least squares estimation can be used. As a method of detecting a rectangle from a contour or an edge, a method of fitting a rectangle so that all the sequences of points in a contour are included, can be used. As such, the shape identification unit **226** is configured to extract a contour of a cell mass from an image picked up in the magnifying image-pickup process and identify a shape thereof.

The shape determination unit **227** is configured to determine whether the cell mass identified by the shape identification unit **226** is in a predetermined shape. The "predetermined shape" is a shape set in advance for a cell mass, and is preferably of the order of such a shape that can be determined to have a high possibility of continuously growing in a manner suitable for observation and is close to a circle to the highest degree possible.

As the criteria for determining a predetermined shape of a cell mass, criteria such as a size and the degree of unevenness may be added to the shape, for example. The criteria for determining a shape includes the degree of ellipse of an ellipse surrounding the contour, roundness of a circle surrounding the contour, for example. The criteria for determining a size includes a size of a white pixel mass, a length of a contour of a white pixel mass, the area of the interior of a contour of a white pixel mass, a length of a long axis of an ellipse, a length of a short axis of an ellipse, a length of a circumference of an ellipse, a diameter of a circle, a length of a circumference of a circle, a length of a rectangle surrounding the contour, the area of a rectangle surrounding the contour, for example. The criteria for determining the degree of unevenness includes the ratio of the area of the contour to a peripheral length, the ratio of the area of the contour to the area of a rectangle surrounding the contour, the ratio of the length of the contour to the length of the rectangle surrounding the contour, the number of corners in a contour, the ratio of the area of the contour to the area of a circle or an ellipse surrounding the contour, the ratio of the length of the contour to the length of a circumference of a circle or a circumference of an ellipse surrounding the contour, the ratio of the area of a rectangle surrounding the contour to the area of a circle or an ellipse surrounding the contour, the ratio of the length of a rectangle surrounding the contour to the length of a circle or an ellipse surrounding the contour, for example. As a method of corner detection when determination is made on the basis of the number of corners in a contour, Harris corner detection, SUSAN operator can be used, for example.

Here, the criteria for determining the predetermined shape of a cell mass is set at the degree of ellipse equal to or smaller than 1.1, for example, and is stored in the storage unit **210**. The degree of ellipse is the ratio of the long-axis length of the ellipse to the short-axis length thereof. As a result, a cell mass close to a circle to the highest degree possible is identified, thereby being able to automatically select a cell mass having an appropriate shape for continuing observation. As a result, it becomes possible to lower the observation priority of a cell mass that has grown in a distorted shape during a growing process, or stop the observation, and thus observation of a cell mass having an appropriate shape can proceed more efficiently.

Further, not only a method of explicitly determining a shape by a threshold value (e.g., the degree of ellipse of 1.1), such a method can be used that cell mass images are sorted based on superiority of determination results and displayed on a monitor **204***a* (in the case of the degree of ellipse, display is made in order from the smaller degree of ellipse), thereby leaving, to a user, the determination of a range in which the cell mass is considered suitable.

The storage unit **210** is configured to store various types of data relating to observation of a cell and an operation of the observation system S, and includes an observation timing holding unit **211**, an observation position holding unit **212**, a position updatability holding unit **213**, a threshold value holding unit **214**, a shape holding unit **215**, and an observation image holding unit **216**, for example.

The observation timing holding unit **211** is configured to hold various types of data relating to time periods and days such as a time period, the number of days, deadlines relating to observation. For example, the data includes: the "predetermined identification time period" that is a predetermined time period relating to identification of a cell mass set with respect to timing for making identification of a magnifying observation target cell mass; "the predetermined number of identification days" that is the predetermined number of days set with respect to timing for finishing identification of a magnifying observation target cell mass; the "predetermined image-pickup period" that is a predetermined time period relating to image pickup of a magnified image set with respect to timing for picking up an image of a magnified cell mass; the "predetermined observation deadline" that is a predetermined deadline set in advance with respect to timing for ending observation of a cell. These types of data are used as criteria for determination as appropriate in the observation program **220** and compared with the time periods and days measured by the time-measuring unit **202**.

The observation position holding unit **212** is configured to hold data such as an observation position (coordinates) of a

cell mass obtained by the entire observation, or an observation position (coordinates) set manually.

The position updatability holding unit **213** is configured to hold a flag indicating whether to update an observation position of a cell mass obtained during the previous entire observation and stored in the observation position holding unit **212**, in accordance with the predetermined identification time period stored in the observation timing holding unit **211**.

The threshold value holding unit **214** is configured to hold various types of data relating to a threshold value with respect to observation. For example, the data includes: a threshold value for determining whether it is a white pixel or a black pixel during the binarization process; a threshold value relating to the number of pixels for determining whether to extract the labeled white pixel cell mass as a cell mass.

Further, the threshold value holding unit **214** is configured to hold a threshold value for identifying a cell mass capable of growing in a favorable manner, when shape identification process is executed. For example, such data includes: a threshold value of the number of pixels for determination by the size of a white pixel mass; a threshold value of the length of a contour for determination by the length of the contour of the white pixel mass; a threshold value of an area for determination by the area of the interior of the contour of the white pixel mass; a threshold value of roundness for determination by the roundness of a circle surrounding the contour; a threshold value of the ratio of an ellipse long axis to an ellipse short axis for determination by the degree of ellipse of an ellipse surrounding the contour; a threshold value of a diameter for determination by the diameter of a circle; a threshold value of a circumference for determination by the length of a circumference; a threshold value of a long axis for determination by a long-axis length of an ellipse; a threshold value of a short axis for determination by a short-axis length of an ellipse; a threshold value of an ellipse circumference for determination by the length of a circumference of an ellipse; a threshold value of a length of a rectangle for determination by the length of the rectangle surrounding the contour; a threshold value of the area of a rectangle for determination by the area of the rectangle surrounding the contour; a threshold value of the ratio of the area of the contour to the peripheral length for determination by the ratio of the area of the contour to the peripheral length; a threshold value of the area ratio for determination by the ratio of the area of the contour to the area of the rectangle surrounding the contour; a threshold value of the length ratio for determination by the ratio of the length of the contour to the length of the rectangle surrounding the contour; a threshold value of the number of corners for determination by the number of corners of a contour; a threshold value of the area ratio for determination by the ratio of the area of the contour to the area of a circle or an ellipse surrounding the contour; a threshold value of the length ratio for determination by the ratio of the length of the contour to the length of a circle or an ellipse surrounding the contour; a threshold value of the area ratio for determination by the ratio of the area of the rectangle surrounding the contour to the area of a circle or an ellipse surrounding the contour; and a threshold value of the length ratio for determination by the ratio of the length of the rectangle surrounding the contour to the length of a circle or an ellipse surrounding the contour.

The shape holding unit **215** is configured to hold a shape identification process result of a cell mass for all the methods of the shape identification process.

The observation image holding unit **216** is configured to hold magnified observation images and entire observation images.

The observation timing holding unit **211** and the threshold value holding unit **214** also function as a setting unit by which a user can change various settings relating to the observation program **220** as appropriate. The setting matters that can be set using the observation timing holding unit **211** include: timing for picking up an image in the entire observation unit **10** or the magnifying observation unit **20**; and the time period, the number of days, the deadline relating to the observation, for example. The setting matters that can be set using the threshold value holding unit **214** include: the predetermined size of a cell mass that is a determination standard of a magnifying observation target cell mass; a predetermined shape of a cell mass that is a determination standard on whether the cell mass is in an appropriate shape for continuing observation, for example.

The time-measuring unit **202** is configured to measure time relating to time periods and days from start of observation of a cell and operation control of the observation system S, thereby being able to grasp various types of time.

The input unit **203** includes pointing devices such as a keyboard **203***a*, a mouse **203***b*, for example. The user inputs characters, numerical values by using the keyboard **203***a*. Further, the user moves a cursor in an arbitrary direction on a screen of the monitor **204***a* of the output unit **204** by using the mouse **203***b*, and selects a menu or other options. The arithmetic unit **201** is configured to execute various types of processes to a program, data, and a file stored/inputted in/to the arithmetic unit **201** and the storage unit **210** on the basis of information obtained from the input unit **203**, and execute an output process to the output unit **204**.

The output unit **204** includes: the monitor **204***a* such as a liquid crystal display, a CRT; and a speaker **204***b*, for example. The arithmetic unit **201** is configured to cause a window, an icon, a menu to be displayed on the monitor **204***a* on the basis of the executed process of a program, and cause sound to be emitted from the speaker **204***b*. Further, the arithmetic unit **201** is configured to cause characters, numerical values inputted by the user to be displayed on the monitor **204***a* on the basis of the information from the input unit **203**, and cause a cursor that is to be moved by the user to be displayed.

Subsequently, an operation of the observation system S by the user relating to observation of a cell in the container C will be described along a flow illustrated in FIG. **6**. FIG. **6** is an explanatory diagram illustrating the flow relating to an operation of the observation system S.

The user first turns on the observation apparatus **1**, the control device **100**, and the computer **200**, thereby starting the observation system S (Step #**101** in FIG. **6**). Then, the user sets the container C containing a cell and a culture fluid for the cell on the holder **31** of the conveying unit **30** (Step #**102**). Subsequently, the user starts the observation program **220** in the computer **200** (Step #**103**), and then an operation screen is displayed on the monitor **204***a*.

The observation program **220** is executed to perform an origin return operation of the conveying unit **30** automatically together with startup of the program (Step #**104**). Then, the observation program **220** is executed to start image pickup by a camera (Step #**105**), and display a real-time image from the camera on the monitor **204***a*.

Subsequently, the user executes a mode setting operation (Step #**106**). In this mode setting operation, a normal time-lapse search operation (Step #**107**) and an entire observation operation (Step #**108**) can be selected. The time lapse observation is a method of observing a position, set in advance, in every predetermined time period.

In the normal time-lapse search operation (Step #**107**), the user observes the interior of the container C while moving the container C using an arrow key on the monitor **204***a* or the keyboard **203***a*, and checks a target cell. Then, the user executes acquisition, display and storage of a captured image, and further setting of coordinates, storage of the coordinates.

In the entire observation operation (Step #**108**), the user sets the predetermined identification time period and the predetermined number of days for identification in the entire observation. The acquisition, display and storage of the image and further observation position display are automatically executed on the basis of the setting.

Subsequently, in an operation according to purpose (Step #**109**), a choice can be made from operations of end (Step #**110**), visual observation continuation (Step #**111**), and the time lapse (Step #**112**).

If the end (step #**110**) is selected, the image pickup by the camera is stopped, and the setting is stored. If the visual observation continuation (Step #**111**) is selected, manual capture storage of an image picked up by the camera can be performed.

If the time-lapse (Step #**112**) is selected, operations of start of the time-lapse observation, time-lapse temporary stop, and time-lapse restart can be performed. If the time lapse is temporarily stopped, works such as taking-out of the container C, replacement of the culture fluid can be performed (Step #**113**).

By performing the time-lapse observation by using such observation program **220**, it is possible to automatically execute such a series of processes that a cell mass having emerged is identified in an image picked up in the entire image-pickup process and the position thereof is specified; and a shape of the cell mass is identified from an image picked up in the magnifying image-pickup process and a cell mass in an appropriate shape for continuing the observation is selected.

Subsequently, an operation relating to the observation process in the observation system S will be described along a flow illustrated in FIG. **7**. FIG. **7** is a flowchart illustrating the operation relating to the observation process in the observation system S.

If the observation program **220** is executed (Start in FIG. **7**), the observation **220** is first executed to determine whether it is in the predetermined identification time period (Step #**201**). With respect to this, the observation program **220** is executed to cause the time-measuring unit **202** to measure, in advance, time periods and days from start of the observation of a cell after seeding thereof and a time period from the previous identification of the magnifying observation target cell mass by using the magnifying observation unit **20**, for example. The predetermined identification time period is a time period set in advance with respect to timing for making identification of the magnifying observation target cell mass, and it is set at one day, for example, and stored in the observation timing holding unit **211** of the storage unit **210**. The predetermined identification time period can be set as appropriate.

If it is not in the predetermined identification time period at Step #**201** (No at Step #**201**), the observation program **220** causes the magnifying observation unit **20** to pick up a magnified image (Step #**202**), and causes the flow of the operations relating to the observation process to be ended (End in FIG. **7**).

Whereas, if it is in the predetermined identification time period at Step #**201** (Yes at Step #**201**), the observation program **220** is executed to send an instruction to the entire observation unit **10** of the observation apparatus **1** to pick up an image of the entire container C, thereby picking up an image of a cell (Step #**203**). Subsequently, the observation program **220** is executed to perform the binarization process of discriminating between a portion that is not a cell mass and a portion that is a cell mass in the picked-up entire image by using a predetermined threshold value (Step #**204**). As a result, binalization is performed such that the portion that is not a cell mass is converted into black and the portion of the cell mass is converted into white.

Further, the observation program **220** is executed to perform erosion/dilation process (Step #**205**). The erosion/dilation process includes: an erosion process that is a process of taking off a white pixel in contact with a black pixel; and to the contrary, a dilation process that is a process of adding a white pixel in contact with the black pixel. In the erosion process, a mass of micro white pixels can be reversed to black pixels, while in the dilation process, a mass of micro black pixels present in a white pixel region can be reversed to white pixels, thereby exerting an effect of removing noise.

Then, the observation program **220** is executed to execute the labeling process (Step #**206**), calculate the number of white pixels in each small region at an arbitrary position set in advance, and identify a mass of white pixels, that is, a cell mass. Further, the observation program **220** is executed to perform sorting in order from a cell having the greater number of pixels in the identified cell masses, that is, the masses of white pixels (Step #**207**).

Subsequently, the observation program **220** is executed to determine whether the largest mass of white pixels (cell mass) is of a size having a threshold value, that is, a size equal to or greater than the predetermined size (Step #**208**). This predetermined size serving as a threshold value is set at 1000 pixels for the number of pixels, for example, and is stored in the threshold value holding unit **214** of the storage unit **210**. If the largest cell mass is of a size smaller than the predetermined size (No at Step #**208**), the observation program **220** is executed to detect arbitrary coordinates of an entire image picked up by the entire observation unit **10**, set it as a magnifying observation position (Step #**209**), and cause the magnifying observation unit **20** to pick up a magnified image (Step #**202**). Then, the observation program **220** is executed to finish a flow for the operations relating to the observation process (End in FIG. **7**).

Whereas, if the largest cell mass is of a size equal to or greater than the predetermined size at Step #**208** (Yes at Step #**208**), the observation program **220** is executed to determine again whether the cell mass selected is of a size equal to or greater than the predetermined size (Step #**210**). If the cell mass is of a size equal to or greater than the predetermined size (Yes at Step #**210**), the observation program **220** is executed to recognize the mass of white pixels (cell mass) as a magnifying observation target cell mass, and detect the coordinates of the center thereof (Step #**211**). Further, the observation program **220** is executed to convert the coordinates of the center thereof into the coordinates for observation of a magnified image (Step #**212**).

Subsequently, the observation program **220** is executed to set the converted coordinates of the center of the cell mass as a magnifying observation position (Step #**213**), and causes the magnifying observation unit **20** to pick up a magnified image (Step #**214**). Then, the observation program **220** is executed to select the subsequent mass of white pixels (cell mass) as a target to be determined on whether or not it is a magnifying observation target cell mass (Step #**215**), and return to Step #**210** to determine whether or not the cell mass is of a size equal to or greater than the predetermined size.

Until the cell mass identified at Step #**206** becomes of a size smaller than the predetermined size (No at Step #**210**), the flow from Step #**210** to Step #**215** is repeated, and the magnifying observation position of the cell mass recognized as the magnifying observation target is set continuously. When the cell mass identified at Step #**206** is of a size smaller than the predetermined size (No at Step #**210**), the observation program **220** is executed to end the flow for the operations relating to the observation process (End in FIG. **7**).

In the time-lapse observation, a position of a cell mass that is to become a magnifying observation target is set manually by the user, or position setting (position specification) of the cell mass is performed from an entire observation result. For the cell mass whose position has been specified, the observation program **220** is executed to send an instruction to the magnifying observation unit **20** in every predetermined image-pickup period, so that the interior of the container C is magnified and an image of the cell is picked up, and the image pickup is stopped on condition that the predetermined observation deadline is reached.

The predetermined observation deadline is a deadline set in advance with respect to timing for finishing the observation of a cell, and is set at 10 days, for example, and stored in the observation timing holding unit **211**. The predetermined observation deadline can be set as appropriate.

As such, from the image obtained by picking up an image of the entire container C, the cell mass is identified and the coordinates thereof are detected, and then magnification is performed with the detected coordinates as the center of magnification, thereby being able to observe the details of the cell mass. Further, the image of a magnified cell is picked up in every predetermined image-pickup period, and when reaching the observation deadline, the image pickup is finished, thereby being able to perform the time-lapse observation continuously from a time of emergence of a cell mass to a time of completion of growth thereof.

According to the configuration of an embodiment of the present invention, in observing a cell being cultured in the container C, it becomes possible to provide the observation apparatus **1** capable of specifying a cell mass having emerged by observing the entire container C, and observe the details of the specified cell mass by magnifying it. Further, it becomes possible to provide the observation program **220**, the observation method, and the observation system S capable of continuous observation of such a specified cell mass from a time of emergence to a time of completion of growth thereof.

<Second Embodiment>

Subsequently, a description will be given, referring to a flow illustrated in FIG. **8**, of an operation in an observation process regarding an observation program according to a second embodiment of the present invention. FIG. **8** is a flowchart illustrating the operation relating to the observation process in the observation program. Since the basic configuration of this embodiment is the same as that of a first embodiment of the present invention described by using FIGS. **1** to **7**, illustration and description of the constituent elements equivalent to those illustrated in a first embodiment of the present invention will be omitted.

When the observation program **220** according to a second embodiment of the present invention is executed (Start in FIG. **8**), the observation program **220** is first executed to determine whether it is in the predetermined identification time period (Step #**301**). When it is in the predetermined identification time period (Yes at Step #**301**), the observation program **220** is executed to send an instruction to the entire observation unit **10** of the observation apparatus **1**, and cause an image of a cell to be picked up by picking up an image of the entire container C (Step #**303**).

Subsequently, the observation program **220** is executed to determine whether or not the predetermined number of days for identification has elapsed since seeding of a cell in the container C (Step #**304**). With respect to this, the observation program **220** is executed to cause the time-measuring unit **202** to measure, in advance, time periods and days from start of the observation of the cell, for example, after the seeding thereof. The predetermined number of days for identification is the number of days set in advance with respect to timing for finishing identification of the magnifying observation target cell mass, is set at five days, for example, and is stored in the observation timing holding unit **211** of the storage unit **210**.

At Step #**304**, if five days, which are the predetermined number of days for identification after the seeding, have elapsed as the time periods and days from start of the observation of the cell, for example (Yes at Step #**304**), the observation program **220** is executed to cause the magnifying observation unit **220** to pick up a magnified image (Step #**302**), and finish a flow for the operations relating to the observation process (End in FIG. **8**). That is, if five days have elapsed since start of observation, identification of a cell mass to be a magnifying observation target is stopped, and the positional information is not updated.

At Step #**304**, if five days, which are the predetermined number of days for identification after the seeding has not elapsed as the time periods and days from start of the observation of the cell, for example (No at Step #**304**), the observation program **220** is executed to execute the binarization process of discriminating between a portion that is not a cell mass and a portion that is a cell mass in a picked up entire image by using the predetermined threshold value (Step #**305**). Hereinafter, since the operation flow from Step #**305** to Step #**316** is equivalent to the operation flow from Step #**204** to Step #**215** in a first embodiment of the present invention, the description thereof will be omitted.

Then, in the time-lapse observation, identification of the magnifying observation target cell mass described above is repeated until the magnifying observation target cell mass can be recognized in every predetermined identification time period, and the identification of the magnifying observation target cell mass is stopped on the condition that the predetermined number of days for identification (five days) has elapsed. The predetermined identification time period is a time period measured by the time-measuring unit **202** that is set in advance with respect to timing for executing the identification of the magnifying observation target cell mass, and is set at one day, for example, and stored in the observation timing holding unit **211** of the storage unit **210**. The predetermined identification time period and the predetermined number of days for identification can be set as appropriate.

As such, the identification of the magnifying observation target cell mass is repeated every predetermined identification time period (one day) until the magnifying observation target cell mass is recognized, that is, a time of emergence of the cell mass is determined, thereby being able to automatically determine a time of emergence of a cell mass. Further, the identification of the magnifying observation target cell mass is stopped after the predetermined number of days for identification (five days) has elapsed from the start of the observation of the cell, thereby being able to perform only the magnifying observation of the cell mass specified in the entire observation and efficiently proceed with observation until completion of growth thereof.

The determination on whether to continue the identification of the magnifying observation target cell mass may be

performed by a method of leaving such determination to a user by providing a window or a user interface through which the user can make setting freely, in addition to a method of automatically performing determination based on the number of days as Step #**304**.

<Third Embodiment>

Subsequently, a description will be given, referring to a flow illustrated in FIGS. **9** and **10**, of an operation in an observation process regarding an observation program according to a third embodiment of the present invention. FIG. **9** is a flowchart illustrating an operation relating to the observation process in the observation program, and FIG. **10** is a continuation of the flowchart illustrating the operation relating to the observation process illustrated in FIG. **9**. Since the basic configuration of an embodiment of the present invention is the same as those of first and second embodiments of the present invention, illustration and description of the constituent elements equivalent to those illustrated in first and second embodiments of the present invention will be omitted.

In a third embodiment of the present invention, since the operation flow from Step #**401** to Step #**410** in FIG. **9** and the operation flow from step #**411** to Step #**413** in FIG. **10** are the same as the operation flows in FIGS. **7** and **8**, the descriptions thereof will be omitted.

The observation program **220** is executed to temporarily set the coordinates of the center of the cell mass coordinate-converted for magnifying observation at Step #**413** in FIG. **10** as a magnifying observation position (Step #**414**). Then, the observation program **220** is executed to send an instruction to the magnifying observation unit **20**, cause the magnifying observation unit **20** to perform magnification with respect to the magnifying observation position in the container C, and pick up an image of the cell (Step #**415**).

Subsequently, the observation program **220** is executed to extract a contour of a cell mass from an image picked up in the magnifying image-pickup process (Step #**416**), and identify the shape (Step #**417**). Then, the observation program **220** is executed to determine whether or not the identified cell mass is in a predetermined shape, that is, the cell mass has the degree of ellipse equal to or smaller than the threshold value (Step #**418**). The degree of ellipse of the threshold value indicating this predetermined shape is set at 1.1, for example, and is stored in the threshold value holding unit **214** in the storage unit **210**. The degree of ellipse of the threshold value indicating the predetermined shape of the cell mass can be set as appropriate. Further, other criteria for determining the predetermined shape of the cell mass, such as roundness, can be also set in place of the degree of ellipse.

At Step #**418**, if the degree of ellipse of the cell mass is equal to or smaller than the threshold value (Yes at Step #**418**), the observation program **220** is executed to formally set the coordinates of the center of the cell mass as a magnifying observation position (Step #**419**). Whereas, if the degree of ellipse of the cell mass exceeds the threshold value (No at Step #**418**), the observation program **220** is executed to delete the temporarily set magnifying observation position (Step #**420**). Then, the observation program **220** is executed to select the subsequent mass of white pixels (cell mass) as a target to be determined on whether it is the magnifying observation target cell mass (Step #**421**), return to Step #**411**, and determine whether or not the selected cell mass is of a size equal to or greater than the predetermined size.

As such, since the cell mass in a predetermined shape is identified, a cell mass in an appropriate shape for continuing observation can be automatically selected. As a result, observation of a cell mass having grown to a distorted shape during the growing process can be stopped, and observation of a cell mass having an appropriate shape can proceed more efficiently.

The determination of the shape of the cell mass at Step #**418** may be made not only by an explicit method using a threshold value (the degree of ellipse of 1.1, for example), but also such a method may be used that cell mass images are sorted on the basis of superiority of determination results and displayed on the monitor **204***a* (in the degree of ellipse, display is made in order from the smaller degree of ellipse), thereby leaving, to a user, the determination of a range in which a cell mass is considered suitable.

<Fourth Embodiment>

Subsequently, an operation in an observation process regarding an observation program according to a fourth embodiment of the present invention will be described along a flow illustrated in FIG. **11** referring to FIGS. **12** to **21** as appropriate. Since the basic configuration according to this embodiment of the present invention is the same as those of first to third embodiments of the present invention, illustration and description of the constituent elements equivalent to those illustrated in first to third embodiments of the present invention will be omitted.

In maintenance and culture of iPS cell, differentiated colonies are removed, in which a state of cell groups has been changed (differentiated) in the center part or peripheral part, and only undifferentiated colonies are continuously cultured. For example, an undifferentiated colony illustrated in FIG. **12** is constituted by only undifferentiated regions, while a differentiated colony (hereinafter referred to as a differentiated colony **1**) illustrated in FIG. **13** has a differentiated region generated in the center part of the colony. Thus, the observation program according to an embodiment of the present invention is executed to select the undifferentiated colony as a cell mass having an appropriate shape for continuing observation as a target of magnifying observation, and not to perform magnifying observation for the differentiated colonies.

FIG. **11** is a flowchart illustrating an operation relating to the observation process in the observation program and is a continuation of the flowchart illustrating the operation relating to the observation process illustrated in FIG. **9**. Thus, the observation program according to an embodiment of the present invention includes the operation flow from Step #**401** to Step #**410** in FIG. **9** and the operation flow from Step #**511** to Step #**522** in FIG. **11**. The description of the operation flow from Step #**401** to Step #**410** in FIG. **9** that is common with a third embodiment of the present invention will be omitted.

At Step #**511**, similarly to Step #**210** in a first embodiment of the present invention, the observation program **220** is executed to determine whether the selected cell mass is of a size equal to or greater than a predetermined size. If the cell mass is of a size equal to or greater than the predetermined size (Yes at Step #**511**), the observation program **220** is executed to execute contour extraction by using the above-described edge extraction filter, contour tracing by using eight-connection search, for example, so as to extract a contour of the cell mass (Step #**512**). Further, the observation program **220** is executed to detect a circle or an ellipse from the extracted figure of the contour by using the above-described Hough transform, generalized Hough transform, for example (Step #**513**) and further detect the center thereof (Step #**514**).

Here, an example of a result obtained by applying the binarization process and erosion/dilation process (Step #**405** and Step #**406** in FIG. **9**) to an image of the undifferentiated colony shown in FIG. **12** is given in FIG. **14**. In FIG. **14**, a mass of white pixels is in an elliptic shape. Whereas, an

example of a result obtained by applying the binarization process and erosion/dilation process to an image of the differentiated colony **1** shown in FIG. **13** is given in FIG. **15**. In FIG. **15**, the mass of white pixels in an elliptic shape contains a mass of black pixels in an elliptic shape smaller than that.

Further, examples of results obtained by performing contour extraction at Step #**512** for these masses of white pixels are indicated by solid lines in FIGS. **16** and **17**, respectively. Furthermore, examples of results obtained by performing circle/ellipse detection at Step #**513** for the figures of these contours are indicated by broken lines in FIGS. **16** and **17**, respectively. Then, examples of results obtained by performing circle/ellipse center detection at Step #**514** for these ellipses are indicated by a symbol "+" in FIGS. **16** and **17**, respectively.

As the result of circle/ellipse detection at Step #**513**, only one circle or ellipse is detected in the undifferentiated colony as illustrated in FIG. **16**. Whereas, in the differentiated colony **1**, two circles or ellipses are detected as illustrated in FIG. **17**, and they are formed in a so-called doughnut shape in which one contains the other. The so-called doughnut shape may include a case where one circle or ellipse is inscribed in the other circle or ellipse.

Subsequently, the observation program **220** is executed to determine a state of the cell mass on the basis of the shape information of the circle or ellipse extracted at Step #**512** to Step #**514** (Step #**515** and Step #**516**).

Specifically, if a plurality of circles or ellipses are detected and a distance between the centers of two of them is equal to or smaller than a predetermined distance (Yes at Step #**515**), it is determined to be a differentiated colony (predetermined state). Even in an undifferentiated colony, double contour lines might be extracted, and a plurality of circles or ellipses having the centers substantially at the same location might be detected. Thus, in an embodiment of the present invention, if a distance between the centers of two circles or ellipses is equal to or smaller than a predetermined distance (Yes at Step #**515**), as well as a difference in radii of them is equal to or greater than the predetermined value (Yes at Step #**516**), it is determined to be a differentiated colony.

Here, assuming that the threshold value Td of a distance between the centers of two circles or ellipses and the threshold value Tr of a radius difference therebetween are equal, if (distance between the centers)≤Td=Tr≤(radius difference), that is, in the case of a so-called doughnut shape in which two circles or ellipses are in such a relationship that one is contained in the other or one is inscribed in the other, it is determined to be a differentiated colony. By setting Td smaller than Tr, the criteria for detecting the so-called doughnut shape can be made strict so that it is determined to be a differentiated colony only if one of the two circles or ellipses contains the other. To the contrary, considering that an image picked up in the entire image-pickup process is used, by setting Td slightly greater than Tr so as to relax the criteria for detecting the so-called doughnut shape, it can be determined to be a differentiated colony including a case where two circles or ellipses are slightly crossed with each other.

Whereas, if only one circle or ellipse is detected (No at Step #**515**), if distances between the centers are all greater than the predetermined distance (No at Step #**515**), or if the radius differences are all smaller than the predetermined value (No at Step #**516**), it is determined to be an undifferentiated colony.

Then, if it is determined to be an undifferentiated colony, the observation program **220** is executed to select the mass of white pixels (cell mass) as a magnifying observation target cell mass, and the subsequent operation flows are the same as Step #**412** to Step #**415**, Step #**419**, and Step #**421** in a third embodiment of the present invention (Step #**517** to Step #**522**). Whereas, if it is determined to be a differentiated colony, it is not selected as the magnifying observation target cell mass, but the subsequent white pixel mass (cell mass) is selected as a target to be determined on whether it is the magnifying observation target cell mass (Step #**522**), the operation returns to Step #**511**, and it is determined whether the selected cell mass is of a size equal to or greater than a predetermined size.

As such, since the differentiated colony is detected as the predetermined shape (so-called doughnut shape), the undifferentiated colony can be automatically selected as a cell mass having an appropriate shape for continuing the observation. As a result, the magnifying observation is not conducted for differentiated colonies, and observation of an undifferentiated colony can proceed more efficiently.

In a differentiated colony illustrated in FIG. **18** (hereinafter referred to as a differentiated colony **2**), for example, a differentiated region is generated in a peripheral part of the colony, and if the binarization process is executed, the colony results in a mass of white pixels constituted by only undifferentiated regions at the center part. In order to prevent the differentiated colony **2** from being selected as a target of the magnified observation, it is only necessary to execute a ternarization process or a conversion process into four-values instead of the binarization process, for example.

FIG. **20** illustrates, as an example, a result obtained by acquiring brightness distribution of each pixel as illustrated in FIG. **19** and applying the ternarization process to an image of the differentiated colony **2** illustrated in FIG. **18**. Further, FIG. **21** illustrates an example of a result obtained by executing the contour extraction for each of the masses of white pixels and gray pixels at Step #**512**, and further executing circle/ellipse detection at Step #**513** and circle/ellipse center detection at Step #**514**. The observation program **220** can be executed to determine the state of the cell mass on the basis of the shape information of the circle or ellipse extracted as such (Step #**515** and Step #**516**).

In an embodiment of the present invention, the differentiated colony is set in a predetermined state where the magnifying observation is not conducted, but as illustrated in FIG. **22**, the predetermined state (Yes at Step #**515** and Yes at Step #**516**) may be used as a magnifying observation target. In this case, the magnifying observation is not conducted for the undifferentiated colony but only the differentiated colony (predetermined state) can be observed efficiently.

As described above, in the observation program according to a fourth embodiment of the present invention, the shape information of the cell mass identified from the image picked up in the entire image-pickup process at Step #**403** is extracted, and the state of the cell mass is determined on the basis of the shape information so that the undifferentiated colony can be automatically selected as a cell mass having an appropriate shape for continuing observation, thereby being able to lower the observation priority of the differentiated colony and stop the observation. The differentiated colony can be also selected as a cell mass having an appropriate shape for continuing observation.

Further, by selecting a magnifying observation target cell mass on the basis of the determination result of the state of the cell mass obtained based on the shape information, the observation of the undifferentiated colony can proceed more efficiently without conducting the magnifying observation for the differentiated colony. The differentiated colony may be used as a magnifying observation target.

Further, in the case where a circle or ellipse is detected from the figure of a contour of a cell mass and a plurality of the circles or ellipses are detected, if a distance between the centers of two of them is equal to or smaller than a predetermined distance, the colony is determined to be a differentiated colony, thereby being able to detect the differentiated colony as a so-called doughnut shape.

Further, if the distance between the centers of the two circles or ellipses is equal to or smaller than a predetermined distance as well as a difference in radii thereof is equal to or greater than the predetermined value, the colony is determined to be a differentiated colony, thereby being able to detect the differentiated colony as a so-called doughnut shape with higher accuracy.

Further, a magnifying observation target cell mass is selected from cell masses each having a size equal to or greater than a predetermined size, thereby being able to determine a time of emergence of a cell mass, and conduct observation continuously from a time of emergence of the cell mass to a time of completion of growth thereof.

The observation process is executed in every predetermined image-pickup period, and when the predetermined observation deadline is reached, the observation process is finished, thereby being able to conduct the time-lapse observation continuously from a time of emergence of the cell mass to a time of completion of growth thereof.

Further, as described above, by picking up an image of the cell in the container C such that an image of the entire container C is picked up using the CMOS camera **12** that is an image-pickup unit of the entire observation unit **10**, the observation program causes the computer to extract shape information of the cell mass that has been identified from an image picked up in the entire image-pickup process, and based on the shape information, the state of the cell mass can be determined.

Further, the magnifying observation target cell mass is selected on the basis of the determination result of the state of the cell mass obtained based on the shape information, thereby being able to magnify a part of the region in the container C and pick up an image of the magnifying observation target cell mass by the CCD camera **24** that is an image-pickup unit of the magnifying observation unit **20**.

For example, in embodiments described above, a description has been given assuming that a single culture container is to be observed, but a plurality of containers may be observed concurrently by using a tray on which a plurality of culture containers can be placed.

Further, in embodiments described above, the CMOS camera **12** is used for the image pickup unit of the entire observation unit **10** and the CCD camera **24** is used for the image pickup unit of the magnifying observation unit **20**, but a type of the camera to be used may be either the CMOS camera or the CCD camera.

Further, in a fourth embodiment of the present invention, by employing Step #**416** to Step #**420** in a third embodiment of the present invention instead of Step #**518**, the shape determination in third and fourth embodiments of the present invention may be used in combination.

The above embodiments of the present invention are simply for facilitating the understanding of the present invention and are not in any way to be construed as limiting the present invention. The present invention may variously be changed or altered without departing from its spirit and encompass equivalents thereof.

What is claimed is:

1. A non-transitory recording medium having an observation program recorded therein, the observation program configured to cause a computer to execute:

an entire image-pickup process of picking up an image of a sample by picking up an image of an entire container containing the sample and a solution;

a sample mass identification process of identifying a sample mass having a plurality of the samples gathering therein, from the image picked up in the entire image-pickup process;

a sample mass determination process of extracting shape information of the sample mass identified in the sample mass identification process, and determining a state of the sample mass based on the shape information;

a coordinate detection process of selecting a magnifying observation target sample mass from the sample masses identified in the sample mass identification process, and detecting coordinates of a center of the magnifying observation target sample mass, based on a determination result of the sample mass determination process; and

a magnifying image-pickup process of performing magnification with the coordinates detected in the coordinate detection process as a center of the magnification, and picking up an image of the magnifying observation target sample mass.

2. The non-transitory recording medium according to claim **1**, wherein

the sample mass determination process includes:

extracting a contour of the sample mass identified in the sample mass identification process, and detecting a circle or an ellipse from a figure of the extracted contour;

determining that the sample mass is in a predetermined state, when a plurality of the circles or the ellipses are detected and a distance between centers of two of the circles or the ellipses is equal to or smaller than a predetermined distance; and

not selecting the sample mass determined to be in the predetermined state in the sample mass determination process, as the magnifying observation target sample mass.

3. The non-transitory recording medium according to claim **1**, wherein

the sample mass determination process includes:

extracting a contour of the sample mass identified in the sample mass identification process, and detecting a circle or an ellipse from a figure of the extracted contour;

determining that the sample mass is in a predetermined state, when a plurality of the circles or the ellipses are detected and a distance between centers of two of the circles or the ellipses is equal to or smaller than a predetermined distance; and

selecting the sample mass determined to be in the predetermined state in the sample mass determination process, as the magnifying observation target sample mass.

4. The non-transitory recording medium according to claim **2**, wherein

the sample mass determination process includes determining that the sample mass is in the predetermined state, when a plurality of the circles or the ellipses are detected and a distance between centers of two of the circles or the ellipses is equal to or smaller than a predetermined distance and a difference in radii of the two of the circles or the ellipses is equal to or greater than a predetermined value.

5. The non-transitory recording medium according to claim 3, wherein

the sample mass determination process includes determining that the sample mass is in the predetermined state, when a plurality of the circles or the ellipses are detected and a distance between centers of two of the circles or the ellipses is equal to or smaller than a predetermined distance and a difference in radii of the two of the circles or the ellipses is equal to or greater than a predetermined value.

6. The non-transitory recording medium according to claim 1, wherein

the coordinate detection process includes selecting the magnifying observation target sample mass from the sample masses each having a size equal to or greater than a predetermined size, among the sample masses identified in the sample mass identification process, based on a determination result of the sample mass determination process.

7. The non-transitory recording medium according to claim 6, wherein

a time-measuring process of measuring time periods and days from start of observation of the sample is further executed; and

the sample mass identification process includes repeating identification of the sample mass in every predetermined time period relating to identification of the sample mass measured in the time-measuring process, and stopping the identification of the sample mass on condition that the predetermined number of days have elapsed.

8. An observation apparatus comprising:

a computer configured to execute an observation program recorded in the non-transitory recording medium according to claim 1;

a first image pickup unit configured to pick up an image of the sample in the container by picking up an image of the entire container; and

a second image pickup unit configured to perform magnification with the coordinates detected in the coordinate detection process as a center of the magnification, and pick up an image of the sample in the container.

9. An observation apparatus comprising:

a computer configured to execute an observation program recorded in the non-transitory recording medium according to claim 2;

a first image pickup unit configured to pick up an image of the sample in the container by picking up an image of the entire container; and

a second image pickup unit configured to perform magnification with the coordinates detected in the coordinate detection process as a center of the magnification, and pick up an image of the sample in the container.

10. An observation apparatus comprising:

a computer configured to execute an observation program recorded in the non-transitory recording medium according to claim 3;

a first image pickup unit configured to pick up an image of the sample in the container by picking up an image of the entire container; and

a second image pickup unit configured to perform magnification with the coordinates detected in the coordinate detection process as a center of the magnification, and pick up an image of the sample in the container.

11. An observation apparatus comprising:

a computer configured to execute an observation program recorded in the non-transitory recording medium according to claim 4;

a first image pickup unit configured to pick up an image of the sample in the container by picking up an image of the entire container; and

a second image pickup unit configured to perform magnification with the coordinates detected in the coordinate detection process as a center of the magnification, and pick up an image of the sample in the container.

12. An observation apparatus comprising:

a computer configured to execute an observation program recorded in the non-transitory recording medium according to claim 5;

a first image pickup unit configured to pick up an image of the sample in the container by picking up an image of the entire container; and

a second image pickup unit configured to perform magnification with the coordinates detected in the coordinate detection process as a center of the magnification, and pick up an image of the sample in the container.

13. An observation apparatus comprising:

a computer configured to execute an observation program recorded in the non-transitory recording medium according to claim 6;

a first image pickup unit configured to pick up an image of the sample in the container by picking up an image of the entire container; and

a second image pickup unit configured to perform magnification with the coordinates detected in the coordinate detection process as a center of the magnification, and pick up an image of the sample in the container.

14. An observation apparatus comprising:

a computer configured to execute an observation program recorded in the non-transitory recording medium according to claim 7;

a first image pickup unit configured to pick up an image of the sample in the container by picking up an image of the entire container; and

a second image pickup unit configured to perform magnification with the coordinates detected in the coordinate detection process as a center of the magnification, and pick up an image of the sample in the container.

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